Study of E-Cadherin Expression in Normal Oral Mucosa in Oral Precancerous Lesions and in Oral Squamous Cell Carcinomas

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ABSTRACT

BACKGROUND
Oral cancer is the 11th most common cancer in the world, and death due to this ranks 6th globally. India has the highest incidence of oral cancer globally and accounts for approximately 30-40% of all other cancers. Squamous cell carcinoma is the most commonly encountered malignancy of the oral cavity. The most important prognostic factor for patients diagnosed with oral squamous cell carcinoma (OSCC) is metastasis to cervical lymph nodes or distant organs.

METHODS
This study was carried out among patients attending ENT OPD in LLR Hospital attached to G.S.V.M. Medical College, Kanpur. A detailed clinical history of present illness including relevant past history was recorded and a complete general examination was done. A total of 100 cases diagnosed in histopathology laboratory between July 2017 and August 2019 were included. Expression of E-cadherin was evaluated in normal oral mucosa, oral premalignant lesions and oral squamous cell carcinomas and was correlated with pathological grade.

RESULTS
There was male preponderance with a male to female ratio of 4.5:1. Maximum cases of oral lesions were encountered in fourth and fifth decades of life. Majority of the cases were tobacco users (85%).

CONCLUSIONS
Correlation of Squamous Cell Carcinoma cases with the expression of intensity of staining with E-Cadherin was found to be significant with p<0.05. There was significant variation of expression of E-cadherin with histopathological dysplasia of the oral precancerous lesions and the tumour differentiation of the oral cancers in comparison with normal oral mucosa. However, there was no correlation between degree of loss of expression of E-cadherin with the degree of dysplasia or the tumour differentiation of oral cancers. There was a variation in the expression of E-cadherin, but its value as a prognostic marker is questionable for which a longitudinal study with larger sample size is required to confirm this conclusion.

KEYWORDS
Oral cancer, Oral precancerous lesions, Squamous cell carcinoma, E-cadherin, Tumour differentiation.
BACKGROUND

Head and neck cancers constitute a fifth of most frequent human tumour diagnosed worldwide and accounts for approximately 400,000 new cases annually. In India, squamous cell carcinoma constitutes more than 90% of the total of head and neck carcinoma with an incidence rate of 12.6 per lakh of the population. Staging and grading of the disease, are two best parameters that predict survival. The success of clinical therapy for head and neck tumours depends upon the status of lymph node metastasis.1

The occurrence of oral squamous cell carcinoma (OSCC) is a multistep procedure involving interactions between several factors such as tobacco-associated intra-oral carcinogens, areca nut, betel quid and consumption of alcohol, and/or viral infections. OSCCs are often preceded by clinically evident lesions often leukoplakia, and the risk of multiple cancers is 5-10 times greater in patients with OSCCs preceded by leukoplakia. These lesions often progress to cancer if left untreated. An average annual transformation rate of cancer progression is 1% has been proposed based on several studies reporting 5% transformation observed in 5 years. In India, over 80% of OSCCs arise from existing oral lesions (OLs). The ability to predict outcome of OLs remains a major challenge for early intervention. Early detection of OLs that will develop into invasive tumours is essential to improve the poor prognosis of oral carcinoma patients.2

Oral cancer is the 11th most common cancer in the world, and death due to it ranks 6th globally. In India, particularly it has the highest incidence of oral cancer globally and accounts for approximately 30-40% of all other cancers. Most invasive oral cavity carcinomas are generally and most of the time preceded by preinvasive stage of oral precancerous lesions. Malignant transformation of oral leukoplakia (OL) is 1-20% over 1-30 years while oral lichen planus (OLP) and oral submucous fibrosis (OSMF) have malignant transformation of 0.2%/year and 7–13% respectively.3

Oral squamous cell carcinoma (OSCC) is one of the most common malignances worldwide, and its most significant risk factors are the use of tobacco and alcohol. This tumour mostly affects men over 40 years old and frequently occurs in the lower lip, tongue, floor of the mouth, soft palate, and the gingival/alveolar ridge.4 Oral squamous cell carcinoma (OSCC) is the most prevalent malignancy in oral cavity, which is a disease found particularly in low income communities and mainly a problem of older men, 90% being in > 45 year age group who are exposed to the known risk factors of tobacco and/or alcohol.5 Head and neck squamous cell carcinoma (HNSSC) is commonly occurring carcinoma with high mortality rates, and approximately 30% arise in the oral cavity, which is characterized by severe degree of local invasiveness and lymph node metastasis. There are approximately 260,000 new cases of oral cavity squamous cell carcinoma (OSCC) and 124,000 deaths worldwide annually. Despite newer modes of therapies, including adjuvant chemotherapy, radiotherapy, and targeted therapy, the overall 5-year survival rate has not improved significantly during the last two decades. Cervical lymph node metastasis is a major determinant of prognosis of OSCC. Approximately 50% of patients were detected with metastasis to regional lymph nodes at the time of diagnosis. Therefore, it is urgent to study the underlying mechanisms of metastasis and evaluate the factors involved in poor prognosis of OSCC.6

E-Cadherin

E-cadherin is a 120 kDa calcium-dependent transmembrane glycoprotein encoded by the CDH1 gene located on chromosome 16q21, and it is expressed in most epithelial cells. E-cadherin has a major role in establishing cell polarity and in maintaining normal tissue architecture. The intracellular domain of E-cadherin is linked to the actin cytoskeleton through its interaction with its cytoplasmic-binding partners, the catenins (α-, β-, and γ-catenin). The E-cadherin/β-catenin complex, therefore, functions as a component of adherent cell–cell junctions that promote cell adhesion. The accumulation of free cytoplasmic β-catenin and/or its translocation to the nucleus (where it acts as a transcriptional activator through its binding with the members of the TCF/LEF-1 family) has been associated with various physiological and pathological processes, including tumour progression.7

METHODS

This is a prospective study carried out between July 2017 – August 2019 among 100 patients attending ENT OPD in LLR Hospital attached to G.S.V.M. Medical College, Kanpur. A detailed clinical history of present illness including important past history was recorded and a complete general examination was done. Out of these 100 cases, 1 case was of normal oral mucosa, 27 cases were of oral precancerous lesions and 72 cases were of oral squamous cell carcinomas.

Inclusion and Exclusion Criteria

All the adequate oral biopsies coming to the department of pathology G.S.V.M. Medical College were included in the study. Inadequate biopsies were excluded from the study. Received samples were examined grossly with respect to number and size of biopsy material. Biopsies were routinely processed. Paraffin blocks were prepared. Paraffin sections were cut. Sections were stained using haematoxylin and eosin stain. A detailed histopathological evaluation was done. E-cadherin staining was done in all cases. Expression of E-cadherin was evaluated in normal oral mucosa, oral precancerous lesions and oral squamous cell carcinomas and was correlated with pathological grade and stage of the tumour.

Selection of Cases

All patients with suspicious premalignant and malignant lesions of oral cavity were worked up with histopathological examination after detailed clinical examination.
Premalignant lesions were selected based on the absence of any invasive squamous cell carcinoma.

**Clinical Assessment**
Detailed clinical history of patients which includes age, sex, duration of symptoms, duration of illness, adverse oral habits like smoking or chewing of tobacco, alcohol consumption, clinical details of the lesion and clinical staging of patients were recorded on a detailed pre-tested structured proforma schedule by interviewing the prospective patients.

**Lab Assessment**
Histological Assessment- Slides prepared after biopsy were stained with haematoxylin and eosin stain and then studied for histological changes. All cases with oral cavity were separated histologically into premalignant and malignant lesions. It was appreciated that all of these changes were not necessarily seen in any one case, and also noted that, minor degree of cellular atypia were seen in some inflammatory conditions. Immunohistochemical Evaluation- Anti-E-cadherin antibody was used.

### RESULTS

<table>
<thead>
<tr>
<th>Grades of Squamous Cell Carcinoma (SCC)</th>
<th>Intensity of E-Cadherin Expression</th>
<th>Total Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+1 (%)</td>
<td>+2 (%)</td>
</tr>
<tr>
<td>Well Differentiated SCC (Grade I)</td>
<td>(39.13)</td>
<td>(47.83)</td>
</tr>
<tr>
<td>Moderately Differentiated SCC (Grade II)</td>
<td>2 (71.43)</td>
<td>8 (22.22)</td>
</tr>
<tr>
<td>Poorly Differentiated SCC (Grade III)</td>
<td>4 (66.67)</td>
<td>2 (33.33)</td>
</tr>
</tbody>
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Table 1. Results of Intensity of E-Cadherin Expression in Different Grades of Squamous Cell Carcinoma

The present study was conducted in the Department of Pathology, GSVM Medical College, Kanpur. The cases for study were collected from ENT OPD in LLR Hospital attached to GSVM Medical College, Kanpur. A total of 100 cases were studied and analysed. Following observations were made- Males predominated over females in a ratio of 4.5:1. Most of the cases belonged to rural areas with a rural urban ratio of 2.7:1. Maximum cases of oral lesions were encountered in fourth and fifth decades of life. The common risk factors observed in patients with oral and oropharyngeal lesions were in the form of tobacco chewing specially gutkha, smoking and taking pan and pan masala. Majority of the cases were tobacco users (85%). Most common site involved was buccal mucosa (25%), followed by tongue (19%) and soft palate (16%). Average duration of presentation was 6 months from the onset of symptoms. One case of normal oral mucosa (NOM) was included in the present study. Oral Precancerous lesions accounted for 27% of the cases and included- Oral submucous fibrosis (29.63%), leukoplakia with moderate dysplasia (22.22%), leukoplakia with severe dysplasia (25.93%) and Verrucous hyperplasia (22.22%). Oral Cancerous lesions accounted for 72% of the cases and included- Well differentiated SCC/Grade I lesion (31.94%), Moderately differentiated SCC/Grade II lesions (48.62%), Poorly differentiated SCC/Grade III lesions (8.33%) and Verrucous Carcinoma (11.11%). The association according to above distribution of cases was found to be highly significant (p<0.001 on applying chi-square test). Intensity of E-Cadherin expression in Oral submucous fibrosis (OSMF) cases scored +2 and +3 in majority of cases. Intensity of E-Cadherin expression in Leukoplakia with moderate dysplasia cases scored +1 in majority of cases. Intensity of E-Cadherin expression in Leukoplakia with severe dysplasia cases scored +1 in majority of cases. Intensity of E-Cadherin expression in Verrucous Hyperplasia cases scored +2 and +3 in majority of cases. Intensity of E-Cadherin expression in Moderately differentiated SCC cases scored +1 in majority of cases. Intensity of E-Cadherin expression in Poorly differentiated SCC cases scored +1 in majority of cases.
DISCUSSION

It was found that the peak incidence was in the fourth and fifth decade of life. Similar findings were reported by two other Indian workers. The reasons for earlier occurrence of carcinoma among Indian people might be habit of tobacco smoking early in life and prevailing poor socio-economic conditions which affect the general nutritional status of the individual.

The risk was from 2.5 to 5 times higher for heavy smokers than for non-smokers. Numerous other epidemiological studies on various Indian population have indicated that tobacco is a vital consideration in the evaluation of tobacco as a risk factor in oral cancer.

The shift in cancer research from the histopathological to the molecular and genetic levels was brought about by many new technologies, one among them is immunohistochemical study and is believed that understanding the molecular basis of cancer will lead to precise diagnosis and better treatment.

In the oral mucosa, epithelium shows adaption to different mechanical demands and maintains its structure by a process of continuous cell renewal and a lot of factors are involved in maintaining the balance between proliferation and differentiation. One among them is E-cad, localized on the surface of epithelial cells in regions of cell-cell contact known as adherens junctions and regulates the cell aggregations. E-cad is also involved in transduction of signals controlling various cellular events, including polarity, differentiation, growth and cellular migration. In normal epithelium, E-cad is expressed in spinous and basal layer expect for the basal surface of the basal layers and superficial layers as a process of continuous renewal of cells.

Loss of E-cad expression has been noted with poorly differentiated morphology in a large number of malignancies such as cervix, esophagus, including head and neck. Hence, the present study was conducted to study the expression of E-Cadherin in normal oral mucosa, in oral precancerous lesions (OSMF, Leukoplakia and Verrucous hyperplasia) and in oral squamous cell carcinomas ( verrucous carcinoma, well differentiated SCC, Moderately differentiated SCC and Poorly differentiated SCC) by immunohistochemistry.

In the present study, 1 case of Normal oral mucosa as a control, was taken. It has expressed 4+ (very strong expression). This control tissue showed intense continuous membranous staining of E-cad in all the epithelial cells of spinous layer and to less extent staining is seen in basal cells and less staining or complete absence in superficial layers of cells which is suggestive of normal desquamation. In the present study, all the cases were of homogenous type of leukoplakia, showing moderate to severe dysplastic changes. These cases, inferences that, there is no significant association between the degree of dysplasia with down-regulation of E-cad expression. This observation of our study was not consistent to various previous studies who concluded that, reduction in the E-cad expression was seen as the degree of dysplasia increases from mild to severe, as the severity increases.

Overall, the majority of cases in Oral Leukoplakia (OL), showed weak and mild membranous staining of E-cad in suprabasal and spinous layer of cells. Hence, there is a reduction in E-cad expression in dysplastic layers of OL. This observation of our study was consistent with the studies conducted by two other studies who stated that atypical features of dysplasia are strongly correlated to the loss of expression of E-cad. In one study it is explained that, there is a variation in the expression of E-cad in the dysplastic epithelium with varying degrees of dysplasia or severity of dysplasia and location of tissue, would suggest that these alterations are the result of the progression of dysplasia and could be a late event, which is suggestive of change toward a cell phenotype with the ability to invade. These explanations are in accordance with our observation in OL group.

Of 8 OSMF cases, majority of cases showed 2+ (mild to moderate expression) and 3+ (moderate to strong expression). In our study, majority of OSMF cases showed reduced expression of E-cad with mild to moderate intensity. This finding of our study was consistent with another study. Expression of E-Cadherin in Oral Verrucous Carcinoma has been reported to be significantly greater than that in poorly differentiated SCC. Our study showed 2+ (mild to moderate expression) and 3+ (moderate to strong expression) staining in majority of the cases of both Oral Verrucous Carcinoma and Oral Verrucous Hyperplasia, indicating that disturbances of adhesion molecules appear to be a property of more severe and advanced neoplasia in this area.
Among cases of Oral Squamous Cell Carcinomas, majority of samples has shown no significant association between the degree of differentiation of tumour and expression of E-cad. This observation of our study was consistent with the other study conducted\(^{16,17}\) whose studies also showed no significant association between the degree of differentiation of tumour and expression of E-cad.

Amongst 23 cases of Well differentiated SCC, majority of the cases scored 2+ (mild to moderate expression). Amongst 35 cases of moderately differentiated SCC, majority of the cases scored 1+ (weak expression). Amongst 6 cases of Poorly differentiated SCC, majority of the cases scored 1+ (weak expression). Majority of samples showed mild to moderate expression, these observations of our study suggest that, there was overall reduction or lost membranous expression of E-cad in the deep invasive tumour margin of primary tumour. Another study conducted\(^{18}\) which concluded that immunoreactivity of E-cad was progressively reduced from normal mucosa followed by oral precancerous lesions and significantly decreased in primary OSCC (58%), which was also coinciding with our findings of E-cad expression.

Upon assessing the expression of E-Cadherin in normal oral mucosa, in oral precancerous lesions (OSMF, Leukoplakia and Verrucous hyperplasia) and in oral squamous cell carcinomas (Verrucous carcinoma, Well differentiated SCC, Moderately differentiated SCC and Poorly differentiated SCC) by immunohistochemistry, following inferences were obtained:

On applying chi-square test, correlation of precancerous lesions with the expression of intensity of staining with E-Cadherin was found to be Non-Significant with p>0.05. On applying chi-square test, correlation of squamous cell carcinoma cases with the expression of intensity of staining with e-cadherin was found to be significant with p<0.05. Above mentioned findings are consistent with the study of\(^3\) which concluded that mild to moderate expression of E-Cadherin staining in majority of cases was suggestive of reduction in dysplastic cells on comparison to normal oral mucosa cells. This variation in expression of E-Cadherin was found to be statistically significant (p<0.001).

In our study the association according to distribution of cases is good with p<0.001 (on applying chi-square test). In our study correlation between clinically diagnosed cases and biopsy proven cases- Sensitivity- 92.10%, Specificity- 87.50%, Predictive value of positive test- 95.89%, Predictive value of negative test- 77.77%, % of false negative- 7.89%, % of false positive- 12.50%, Odds ratio- 81.66, Relative risk (Prevalence ratio)- 4.31. On applying chi-square test p value was found to be less than 0.0001 (p<0.0001) which showed highly significant association.

**CONCLUSIONS**

There was significant variation in the expression of E-cadherin with regard to the histopathological dysplasia of the oral precancerous lesions and the tumour differentiation of the oral cancers in comparison with normal oral mucosa (NOM). However, there was no correlation of the degree of loss of expression of E-cadherin with the degree of dysplasia or the tumour differentiation of oral cancers. Since, there was a variation in the expression of E-cadherin, but its value as a prognostic marker is questionable for which a longitudinal study with larger sample size is required to confirm this conclusion.

**REFERENCES**


