Original Article

Immunohistochemical expression of E-Cadherin and Cyclin D1 in different grades of oral squamous cell carcinoma

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80–90% of all malignant neoplasms of the oral cavity. It results from the multistep accumulation of heterogeneous genetic changes. Important risk factors for OSCC include the use of tobacco or betel quid chewing, alcohol consumption, human papillomavirus and poor nutrition. E-Cadherin as a tumour suppressor gene sets a threshold for Wnt/β-catenin signalling. When expression of E-Cadherin is lost, potentiation of Wnt signalling pathway occurs leading to loss of cell–cell adhesion. The cyclin D1 gene (CCND1) located on chromosome 11q13 encodes a nuclear protein that is the regulatory subunit of Cdk-4 and Cdk-6. Cyclin D1 plays a major role in cell cycle transition from G1 to S phase by contributing to inactivation of the retinoblastoma gene product, and overexpression of CCND1 has been reported in 35–40% cases of OSCC.

Aim: Considering this, we decided to evaluate and compare the expression of CE-Cadherin and Cyclin D1 in different grades of OSCC.

Materials and Methods: A retrospective study was carried out on 60 formalin-fixed paraffin embedded tissue blocks comprising of 20 cases of well-differentiated OSCC, 20 cases of moderately differentiated OSCC and 20 cases of poorly differentiated OSCC. Diagnosed (using H and E), with oral mucosa taken as control.

Results: There was downregulation of E-Cadherin and overexpression of Cyclin D1 in increasing grades of OSCC and the difference was statistically significant. E-Cadherin was localised to membranous and shifted to cytoplasm as the grade worsened. Cyclin D1 was localised to nuclei of cells and the expression was seen more at the peripheral portions of tumour islands depicting the proliferative activity of tumour front.

Conclusion: The study revealed a good prognostic role of both E-Cadherin and Cyclin D1 in OSCC. The markers can be used for prognostic as well as therapeutic purposes.

Keywords: Cyclin-D1, E-Cadherin, IHC, OSCC

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80–90% of all malignant neoplasms of the oral cavity. It is defined as 'A malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or the presence of intercellular bridges' (Pindborg JJ *et al.*, 1997).[1] Globally, oral cancer ranks sixth among

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all types of cancer. India has the largest number of oral cancer cases and one-third of the total burden of oral cancer globally.[2] Oral squamous cell carcinoma results from the multistep accumulation of heterogeneous genetic changes.[3] Important risk factors for OSCC include the use of tobacco or betel quid chewing, alcohol consumption, human papillomavirus and poor nutrition.[4] The current clinical gold standard for predicting the cancer progression risk for oral potentially malignant disorders (OMPDs) requires biopsy and microscopic evaluation of H and E stained tissue to determine the presence and grade of dysplasia. Despite ubiquitous use, dysplasia is an imperfect risk marker because at its core, carcinogenesis is driven by the accumulation of somatic mutations and epigenetic changes.[3] Therefore much effort has been devoted to the discovery of molecular biomarkers to assess the progression of the lesion.

Tumour markers are the substances that not only help in detecting the malignancy but also differentiate the nature of malignancy involved. Many molecular markers are associated with the occurrence, progression and prognosis of carcinoma. Markers of increased proliferation in oral potentially malignant disorders and oral cancer have been identified and explored for more than a decade.[5]

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.[6] In normal cells, E-Cadherin exerts its tumour-suppressing role mainly by sequestering β-Catenin from its binding to LEF (Lymphoid enhancer factor)/TCF (T cell factor). β-Catenin serves the function of transcribing genes of the proliferative Wnt signalling pathway. E-Cadherin function can be altered at genetic and epigenetic levels.[7]

Cyclin D1, a 45 kDa protein encoded by cyclin D1 gene (CCND1) located on chromosome 11q13, is a part of the molecular system that regulates the cell cycle G1 to S transition. Overexpression of cyclin D1 leads to shortening of G1 phase and less dependency on growth factors resulting in abnormal cell proliferation which in turn might favor the occurrence of additional genetic lesions.[8] With this background, this study was undertaken to evaluate the prognostic role of E-Cadherin and CyclinD1 in OSCC.

MATERIALS AND METHODS

A retrospective study was carried out on 60 formalin-fixed paraffin embedded tissue blocks comprising of 20 cases of well-differentiated OSCC, 20 cases of moderately differentiated OSCC and 20 cases of poorly differentiated OSCC. Diagnosed (using H and E), with normal oral mucosa taken as control.

From each formalin fixed paraffin embedded (FFPE) tissue block, 3–4 μ thick sections were cut and stained by H and E stain for histopathological grading. Tumours were graded according to Broder's criteria.[9] Immunohistochemical study was carried out using the polymer-labelling technique. Sections were dewaxed, washed in alcohol and antigen retrieval was carried out in a Decloaking Chamber with 10 mM Citra solution at 125°C for 30 seconds followed by 90°C for 10 seconds. Slides were cooled naturally and brought to room temperature. Slides were placed inside the autostainer. Endogenous peroxidase was blocked by using 0.3% hydrogen peroxide in methanol at room temperature for 10 minutes. Slides were washed with phosphate-buffered saline (PBS) briefly and incubated with primary antibody (Cyclin D1) and (E-Cadherin) for 60 minutes. Sections were again washed with PBS, incubated with the polymer for 30 minutes, and washed again with PBS. Diaminobenzidine was used as the chromogen in hydrogen peroxide for 10 minutes. Sections were then counterstained with haematoxylin, mounted and studied under a light microscope for immunoreactivity.

Presence of brown-coloured end product at the site of target antigen was indicative of positive immunoreactivity.

In each section, five light microscopic fields (200 × magnifications) were randomly selected. Two observers individually noted the intensity of staining percentage of staining of E-Cadherin and Cyclin D1 in each field and were scored as:

A. Intensity of staining
- Score 0 = No Staining
- Score 1 = Mild Staining
- Score 2 = Moderate Staining
- Score 3 = Intense Staining

B. Percentage of staining
- Score 0 = No staining of cells in any microscopic field
- Score 1+ = Less than 10% of tissue-stained positive
- Score 2+ =10–50% of tissue-stained positive
- Score 3+ =50–80% of tissue-stained positive
- Score 4+ = More than 80% of tissue stained positive

Final IHC scoring:

Immunoreactive score (IRS) was obtained by the product of percentage score (0–4) and intensity score (0–3). A final score was assessed as;

Score 0–1 = Negative
Score 2–3 = Mild
Score 4–8 = Moderate
Score 9–12 = Strongly Positive

Annova test was used to compare the variables and a *P* value of less than 0.05 was considered statistically significant.

RESULTS AND OBSERVATIONS

The expression of E-Cadherin in normal oral mucosa and different grades of OSCC is given in Figure 1. There was strong membranous expression of E-Cadherin in normal oral mucosa and the expression declined in OSCC. Mild to moderate intensity was seen in moderately differentiated squamous cell carcinoma (MDSCC), mild in well differentiated squamous cell carcinoma (WDSCC) and mild to absent in poorly differentiated squamous cell carcinoma (PDSCC) [Tables 1 and 2]. The difference was statistically significant. There was a shift of marker from membranous to cytoplasmic as the grade worsened. Final IRS was obtained by multiplying intensity score and percentage score. Figure 2 shows the final IRS. The figure displays the linear decline in the final IRS; it was strongly positive in normal oral mucosa, moderate in WDSCC, mild in MDSCC and negative in PDSCC.

The expression of Cyclin D1 in normal oral mucosa and different grades of OSCC is shown in Figure 3. Cyclin D1 showed an upward trend in the expression in OSCC. It was mild to absent in normal oral mucosa and the expression was upregulated is higher grades of OSCC [Tables 3 and 4]. There was a strong nuclear expression seen in OSCC especially in MDSCC and PDSCC. The intensity was higher in the peripheral portions of tumour islands which depicts the proliferative potential of tumour cells at the front. The expression was focal heterogeneous in WDSCC, reduced homogeneous in MDSCC and strong homogenous in PDSCC. Figure 4 shows the final IRS of Cyclin D1. The figure displays an upward trend in the expression of marker with highest expression in PDSCC. PDSCC showed an IRS score of 10, MDSCC showed a score of 6, WDSCC showed a score of 3, and normal oral mucosa had a score of 1.

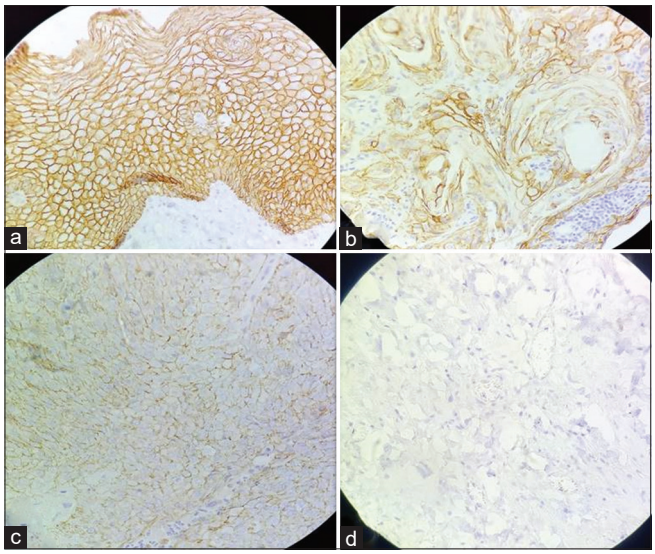

Figure 1: E-Cadherin expression in normal oral mucosa (a) well-differentiated squamous cell carcinoma (b) moderately differentiated squamous cell carcinoma (c) and poorly differentiated squamous cell carcinoma (d)

DISCUSSION

Oral squamous cell carcinoma represents over 90% of malignancies of the oral cavity. The development of oral squamous cell cancer is a multistep process involving the accumulation of multiple genetic alterations modulated by genetic pre-disposition and environmental influences such as tobacco and alcohol use, chronic inflammation and viral infections.[3] All of these factors can lead to a wide range of genetic and molecular alterations that can be detected

Table 1: Intensity of staining of E-Cadherin in various groups

Intensity of staining	NOM	WDSCC	MDSCC	PDSCC
Score 0	0	1	5	11
Score 1	0	8	10	9
Score 2	01	6	3	0
Score 3	04	5	2	0
Total	05	20	20	20

F-statistic value=13.1957, *P*=0.00002

Table 2: Percentage of staining of E-Cadherin in various groups

Percentage of staining	NOM	WDSCC	MDSCC	PDSCC
Score 0	00	1	6	15
Score 1	00	4	5	2
Score 2	01	3	4	2
Score 3	01	4	3	01
Score 4	03	8	2	0
Total	05	20	20	20

F-statistic value=25, *P*=0.00001

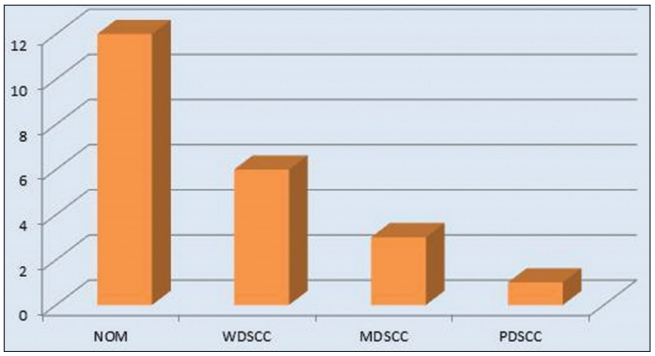

Figure 2: The final immunoreactive score was strongly positive in normal oral mucosa, moderate in WDSCC, mild in MDSCC and negative in PDSCC

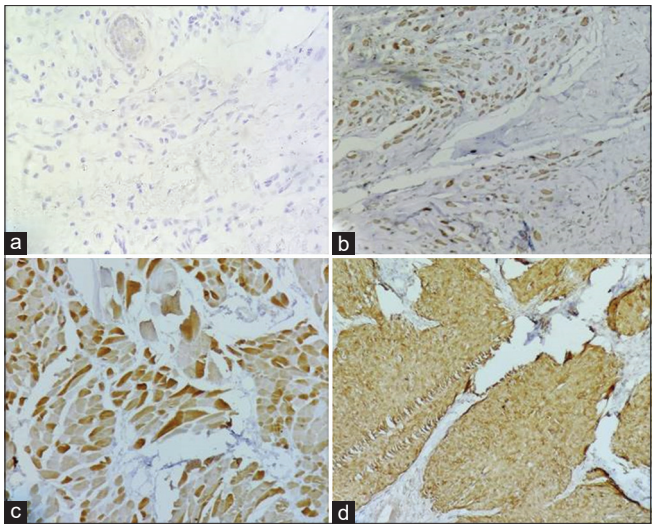

Figure 3: Cyclin D1 expression in normal oral mucosa (a) well-differentiated squamous cell carcinoma (b) moderately differentiated squamous cell carcinoma (c) and poorly differentiated squamous cell carcinoma (d)

using a range of molecular studies. The alterations mostly affect two large groups of genes: oncogenes and tumour suppressor genes, which can be either inactivated or overexpressed through mutations, loss of heterozygosity, deletions or epigenetic modifications such as methylation.[10] Tumour markers are substances that are produced either by the tumour itself or by the body in response to the presence of cancer or certain benign (noncancerous) conditions that can aid in the diagnosis of cancer and in the assessment of tumour burden. Tumour markers not only help in detecting the malignancy but also differentiate the nature of malignancy involved. The amount of their production depends on the growth of tumour cells. Many molecular markers are associated with the occurrence, progression, and prognosis of carcinoma. Markers of increased proliferation in oral potentially malignant disorders and oral cancer have been identified and explored for more than a decade.[5]

Through this study, we made an attempt to evaluate the prognostic role of E-Cadherin and Cyclin D1 in different grades of OSCC.

In normal oral mucosa, there was strong membranous expression of E-cadherin. The staining intensity was moderate to intense and more than 80% of tissue was positively stained. The staining was intense for basal and spinous cells except for basal surface of basal cells and superficial cells. There was loss of staining of E-Cadherin with an increase in the grade of carcinomas. WDSCC showed greater expression of E-Cadherin than MDSCC while PDSCC showed the least expression and the difference was highly significant ($P = 0.0005$).

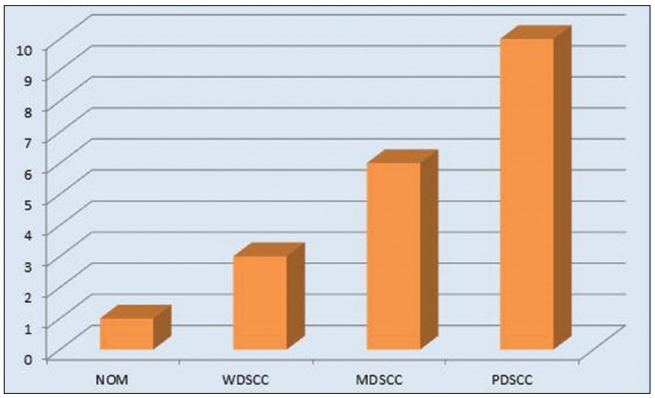

Figure 4: The graph displays an upward trend in the expression of marker with highest expression in PDSCC and least in normal oral mucosa

Table 3: Intensity of staining of Cyclin D1 in various groups

Intensity of staining	NOM	WDSCC	MDSCC	PDSCC
Score 0	02	8	3	1
Score 1	03	6	1	1
Score 2	00	5	8	6
Score 3	00	1	8	12
Total	05	20	20	20

F-statistic value=12.09, $P<0.05$

Table 4: Percentage of staining of Cyclin D1 in various groups

Percentage of staining	NOM	WDSCC	MDSCC	PDSCC
Score 0	01	2	0	0
Score 1	02	2	2	1
Score 2	02	7	5	3
Score 3	00	6	5	6
Score 4	00	3	8	10
Total	05	20	20	20

F-statistic value=6.72, $P=0.0005$

Our findings were consistent with the observations of Kaur G *et al*.[11] (2009), Sridevi U *et al*.[12] (2015), Akhtar K *et al*.[13] (2016), Kushwaha SS *et al*.[14] (2019), and Bankfalvi A *et al*.[15] (2002). Kaur G *et al*.[11] (2009) reported strong expression of E-Cadherin in 90% of the cases of WDSCC and 92.90% of the cases of MDSCC. 69% PDSCC cases showed weak staining while 15% cases showed strong staining and 15% cases exhibited negative staining. The localisation was seen to be membranous in WDSCC that shifted to cytoplasmic in MDSCC and PDSCC. Sridevi U *et al*.[12] (2015) reported weak expression in all the cases of WDSCC. There was loss of expression in 66%, weak expression in 33% and strong expression in 33% cases of MDSCC. Loss of expression was seen in 50% and weak expression in 50% of cases of PDSCC. Akhtar K *et al*.[13] (2016) reported weak expression in 60%, strong expression in 30% and loss of expression in 10% of the cases of WDSCC; loss of expression in 80% and weak expression in 20% of the cases of MDSCC; loss of expression in 90% and weak expression in 10% of the

cases of PDSCC. Kushwaha SS *et al*.[14] (2019) in their study found that well-differentiated squamous cell carcinoma showed greater expression of E-Cadherin than moderately differentiated OSCC, poorly differentiated OSCC showing the least expression.

In the present study, in normal mucosa 60% of the cases showed mild staining intensity of Cyclin D1 in the nucleus of basal cells and few cells in the parabasal layer which is similar to the study results of Swaminathan U *et al*.[16] (2012), Angadi PV and Krishnapillai R[17] (2007).

In WDSCC, mild to moderate staining was seen which is consistent with the findings of Patel SB *et al*.,[18] Angadi PV and Krishnapillai R[17] and Goto H *et al*.[19] Our results are in contrast to the results of Ohnishi Y *et al*.[20] in which 90% of WDSCC showed strong staining of cyclin D1. In MDSCC, 50% of cases showed intense staining for cyclin D1 while 50% of cases showed moderate staining which is nearly similar to the observations of Swaminathan U *et al*.[16] This is in contrast to the study of Angadi PV and Krishnapillai R[17] who reported mild to moderate staining intensity in MDSCC. In our study, intense staining was observed in PDSCC. The difference between the mean scores of intensity of staining of cyclin D1 between the study groups was found to be statistically significant. Cyclin D1 was expressed in the outer layers of the epithelial tumour islands and cords since cyclin D1 is an activator of the cell proliferation cycle and peripheral cells are those which are supposed to be the most proliferative and invasive ones in OSCC.

CONCLUSION

In conclusion, this study reports that alteration of E-Cadherin and cyclin D1 is frequent in OSCC. In our study, expression of E-Cadherin and cyclin D1 was significantly altered in different grades of OSCC. This indicates and supports the previous studies that overexpression of cyclin D1 and downregulation of E-Cadherin may be an early event in oral cancer development.

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Conflicts of interest
There are no conflicts of interest.

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