Original Article

Evaluation of e-cadherin and vimentin expression for different grades of oral epithelial dysplasia and oral squamous cell carcinoma – An immunohistochemical study

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Abstract

Background: Oral cancer is the 11th common cancer in the world and ranks 6th globally in deaths. The incidence of oral cancer in India accounts for approximately 30%–40% of all cancers.

Aims and Objective: The present study was undertaken to evaluate the expression of Vimentin and E-cadherin in different grades of oral epithelial dysplasias (OEDs) and oral squamous cell carcinoma (OSCC).

Materials and Methods: Biopsies/blocks of oral cavity lesions were retrieved from the archives of the department. Normal oral mucosa (5 cases), oral epithelial dysplastic (60 cases) and different grades of OSCC (60 cases) evaluated by hematoxylin and eosin sections. Immunohistochemical analysis was done on the blocks and expression of E-cadherin and Vimentin was recorded.

Results: Our study included various grades of OED, OSCC and normal mucosa as control cases. The mean age of OED and OSCC was 49 and 56 years, respectively, with male predominance. Tobacco habit was present in approximately 90% cases, and buccal mucosa was the most commonly involved site in oral cavity with whitish patch and ulceroproliferative lesions being the common clinical presentations respectively. In OED, downregulation and altered localization of e-cadherin (81.6%) and increased expression of vimentin (52.3%) along with their concurrent increase in the stroma represent epithelial mesenchymal transition. In OSCC, reduction in expression (<50%) for e-cadherin (56.6%) with altered localization for e-cadherin was seen in 88.3% of OSCC along with neoexpression of vimentin in the epithelial cells was seen in 68.3% suggestive of mesenchymal phenotypic modification ($P = 0.05$).

Conclusion: It is very crucial to evaluate the invasiveness of dysplasia and tumor with specific molecular biomarker that may help in early prediction of malignancy and also guide in deciding best treatment strategy for established cases of malignancy.

Keywords: Buccal mucosa, e-cadherin, immunohistochemistry, oral epithelial dysplasia, squamous cell carcinoma, vimentin

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most frequent malignancy of oral cavity ranked the 6th most common cancer worldwide causing deaths.[1,2] Its association with chronic tobacco/areca nut use is particularly more

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prevalent in the Indian subcontinent.[3] Despite the advent of newer diagnostic and therapeutic approaches, the overall 5-year survival rate is poor (<50%) making it a global health problem.[4,5] The management is often associated with severe functional and cosmetic defects leading to substantial morbidity.[4]

Progression of these oral potentially malignant disorders to carcinoma is multi-stage process wherein the normal mucosa on exposure to tobacco/areca nut can progress to the development of premalignant lesions, i.e., oral leukoplakia, erythroplakia and oral submucous fibrosis (OSMF) which can further proceed to malignancy.[2] OSCCs have a propensity to metastasize to the loco-regional lymph nodes as well as to distant sites resulting in considerable disability and mortality.[6] Despite the fact that the mucosa may appear clinically normal, there may be changes at the molecular level predisposing the development of potentially malignant lesions and OSCC. Currently, histological grade is the best predictor for progression of premalignancy to malignancy and to define the aggressiveness of cancer.[7] Markers of epithelial mesenchymal transition (EMT) can help in early detection and management of OSCC in the premalignant stage, and can considerably improve the cancer free survival and quality of life of the patients.[8]

Aims and objectives

The aim and objective were to evaluate the expression of e-cadherin and vimentin in different grades of oral epithelial dysplasia (OED) and OSCCs. The correlation of e-cadherin and vimentin between normal mucosa, dysplasia, OSCCs with vimentin and e-cadherin was done.

MATERIALS AND METHODS

Patients and specimens

A total of 125 cases were included in our study, and the blocks for the period of 2 years (2017–2019) were retrieved from department archives of our institute. The cases were biopsies of normal buccal mucosa (5 cases), OED (mild, moderate, severe dysplasia 20 cases each) and OSCC (well, moderate and poorly differentiated 20 cases each). None of the patients had received any tumor-specific therapy (chemotherapy or radiotherapy). The study was approved by the institutional ethical committee.

Histopathology diagnosis

Tissues blocks were retrieved from department repository. Sections were cut at 4μm and stained with H and E. Histological grading was done on hematoxylin and eosin-stained sections according to the revised criteria given by the World Health Organization 2017 for OED, whereas grading of squamous cell carcinoma was based on Bryne's invasive front grading system.[1,9]

Immunohistochemistry

The tissue sections (4 μm) were cut from representative paraffin blocks collected in pre-coated poly-l-lysine slides and were then deparaffinized in xylene and rehydrated in graded alcohol. Endogenous peroxidase was blocked using 3% hydrogen peroxide. Antigen retrieval was done by conventional microwave method in 10 mMol/l sodium citrate retrieval buffer (pH 6.0) for 10-, 10- and 5-min slots. The sections were then incubated with following steps-super enhancer 20 min, 3 times wash with buffer solution, primary antibodies 45 min (antibodies used were e-cadherin, clone A36-Mouse monoclonal IgG1, Biogenex Code No: AM390-5M and Vimentin, clone V9-Mouse Monoclonal IgG, Biogenex Code No: AM074-5M ready to use) 3 times wash with buffer solution, secondary antibody kit (histidine-rich protein) for 20 min, 3 times wash with buffer solution, DAB stain for 1 min and 3 times wash with buffer solution, followed by counterstain with hematoxylin.

Evaluation of immunoreactivity

IHC stained slides were semi-qualitatively evaluated on the basis of staining of epithelial cells or mesenchymal. The antibodies expression was studied using the following four parameters:

1. Intensity
2. Location
3. Percentage positivity
4. Extent.

The expression of the epithelial proteins like e-cadherin was evaluated only in the epithelial cells, while the expression of the mesenchymal proteins like vimentin was evaluated both in the cells of the epithelium and the connective tissue. The parameters 1st, 2nd and 3rd criteria were same for E-cadherin and vimentin while the 4th parameter criteria varied based on the antibody and its expression in epithelium or connective tissue.

Statistical analysis

The correlation between clinicopathological parameters and e-cadherin and vimentin expression was analyzed using the Chi-square test and Fisher's exact test. A $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathological features

The study population was taken from the archives of the department records. A total of 125 cases included

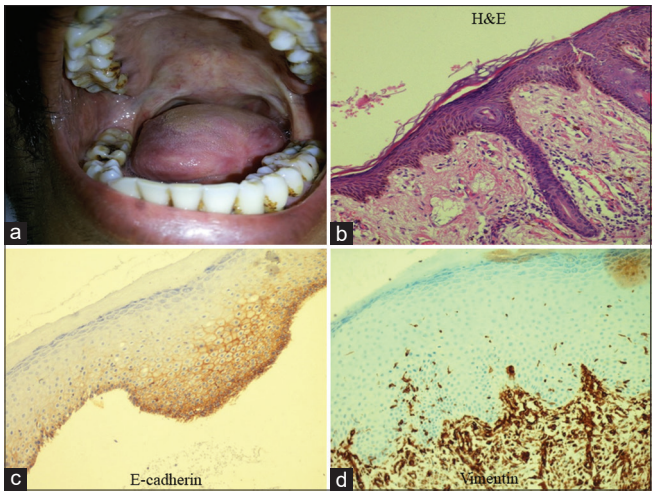

Figure 1: (a) Clinical picture of normal buccal mucosa; (b) Histopathology showing the normal buccal mucosa (H&E, ×40); (c) E-cadherin showing intense membranous staining in normal lower 1/3rd mucosal epithelium; (d) Vimentin showing negative expression in normal mucosal epithelium and intense positivity in the connective tissue (IHC, ×20)

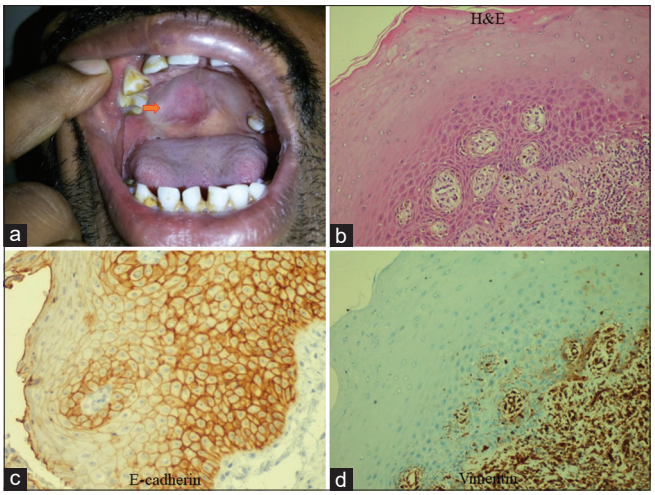

Figure 2: (a) Clinical picture for whitish patch over roof of hard palate; (b) Histopathology showing the variable acanthosis with mild basal layer atypia (H&E, ×40); (c) E-cadherin is predominantly membranous with mild reduction in intensity of stain; (d) Vimentin is focal mild intensity expression in cytoplasmic epithelium (IHC, ×20)

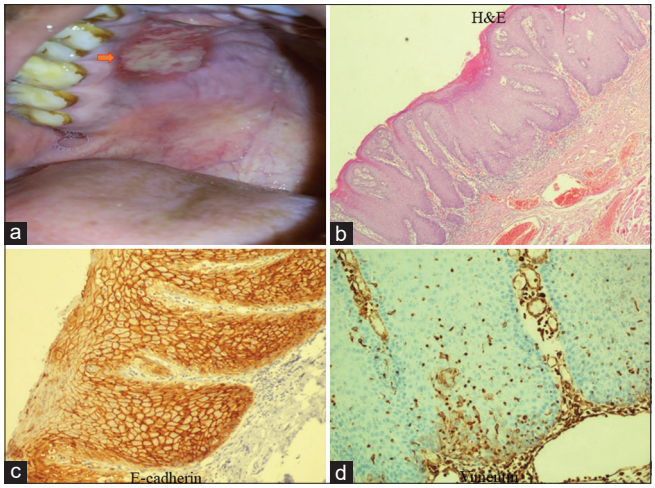

Figure 3: (a) Clinical picture of patient with erythema and central whitish plaque in the roof of hard palate; (b) Epidermis with acanthosis and papillomatosis and moderate degree of atypia in >1/3rd thickness (H&E, x20); (c) E-cadherin with moderate intensity staining extending up to stratum corneal with membranous and cytoplasmic expression; (d) Vimentin showing mild expression in basal layers (IHC, x20)

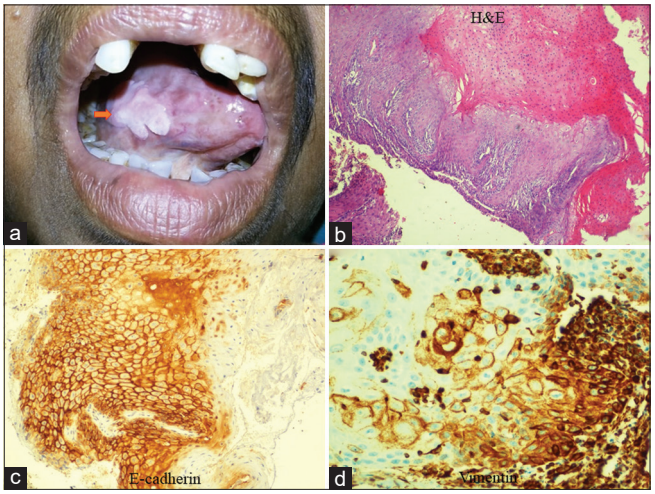

Figure 4: (a) Clinical picture of patient with verrucous plague over lateral border of tongue; (b) Epidermis with full-thickness dysplasia and nuclear atypia (H&E, ×20); (c) E-cadherin intense expression extending up to stratum granulosum with membranous and cytoplasmic pattern; (d) Vimentin showing moderate intensity in basal layers of epidermis (IHC, x20)

5 normal cases, 60 OED and 60 OSCCs. OED was predominantly in 4th decade age group (48.3% cases) with a mean age being 49.05 years. On the contrary, OSCC was more predominant in the age 5th to 6th decade (43.4% cases) with a mean age of 56 years.

OED and OSCC were more predominant among the males with a ratio of 2:1 and 3:1, respectively. History of tobacco consumption was present in OED and OSCC in 93% and 83%, respectively, however the details of smoking history/tobacco type and number of pack consumption/year was not avaliable in the retrieved records. Buccal mucosa was the most common site to be involved in all the study groups (63% cases in OED and 43% cases in OSCC) followed by tongue in OED, retromolar trigone in OSCC. The various other sites involved in OED and OSCC included alveolus, tongue, gingiva, lip, floor of the mouth, angle of mouth, palate as well as multiple site involvement. Clinical presentation was whitish patch over buccal mucosa in 42% cases, whereas ulceroproliferative lesion was noted in only 28% cases of OED whereas in OSCC 83% cases.

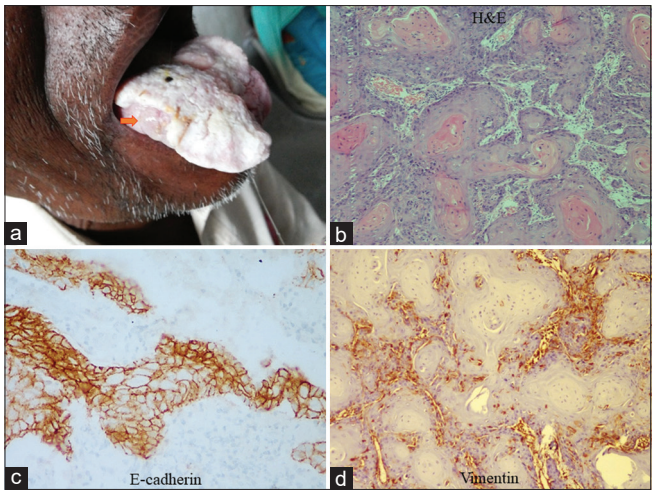

Figure 5: (a) Clinical picture of patient with verrucous lesion extending all over the tongue surface; (b) Histopathology with features of well-differentiated squamous cell carcinoma (H&E, ×40); (c) E-cadherin showing intense membranous and cytoplasmic positivity in >50% of epithelial cells; (d) Vimentin showing mild to moderate expression with infiltrative pattern (IHC, ×40)

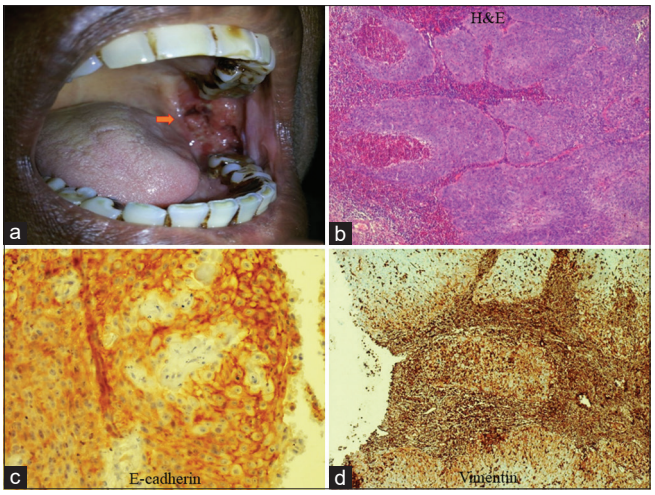

Figure 6: (a) Clinical picture of patient with ulceroproliferative lesion near the retromolar trigone region; (b) Histopathology showing features of moderately differentiated squamous cell carcinoma (H&E, ×40); (c) E-cadherin showing mild-moderate intensity in membrane and cytoplasmic localization of epithelial cells; (d) Vimentin showing moderate to marked cytoplasmic positivity in approx. 50% of epithelial cells (IHC, x20 & x40)

Expression of e-cadherin in oral epithelial dysplasia and oral squamous cell carcinoma patients

E-cadherin expression was predominantly intense for normal mucosa (100%) [Figure 1a-c]. We had a progressive reduction in the intensity of e-cadherin expression over OED grades: mild (90%), moderate (85%) and severe OED (70%) [Figure 2a-c, 3a-c, 4a-c]. The reduction in the intensity of staining pattern was observed with the increasing grades of OSCC well (60%), moderate (45%) and poorly (25%). $P \approx 0.05$ was statistically significant. The normal mucosa was showing membranous pattern of e-cadherin expression (100%). There was a progressive cytoplasmic shift as evident with increasing grades of OED, i.e., mild (5%), moderate (15%) and severe OED (60%) which was statistically significant ($P = 0.001$). Cytoplasmic localization was prominently increasing in the carcinoma group with well differentiated 70%, moderately differentiated 95% and all the poorly differentiated SCCs showing a cytoplasmic location (100%) and statistically significant ($P = 0.008$) [Figure 5a-c, 6a-c, 7a-c].

E-cadherin expression was noted predominantly in the basal/parabasal layer (80%) and spinous layer (20%) of normal mucosa. A progressive involvement of all layers was seen in the OED group with 5% of mild OED, 10% of moderate and 70% of severe OED showing expression till the surface layers ($P < 0.001$) which was statistically significant. This pattern of involvement could not be noted among the OSCC due to lack of intact epithelium in the malignant group.

Vimentin expression in oral epithelial dysplasia and oral squamous cell carcinoma patients

The normal mucosa specimens did not show vimentin expression in the epithelium [Figure 1d]. Vimentin positivity was low in mild OED (45%) while high expression was evident in 15% of moderate and 40% of severe OED, which was statistically significant ($P = 0.02$) [Figure 2d, 3d, 4d]. A progressive high expression (>50%) was evidenced with poor histologic grades, i.e., well-differentiated squamous cell carcinoma (WDSCC) (10%), moderately differentiated squamous cell carcinoma (MDSCC) (20%) and poorly differentiated squamous cell carcinoma (PDSCC) (50%) which was statistically significant ($P = 0.03$). An intense expression was associated with increased grade of OED, i.e., mild (20%), moderate (30%) and severe (60%) so was in OSCC though more pronounced, i.e., WDSCC (40%), MDSCC (60%), PDSCC (85%) which was statistically significant ($P < 0.05$). All cases positive for vimentin in the epithelium were seen to be localized in the cytoplasm.

The connective tissue expression of vimentin was seen in all cases of normal mucosa [Figure 1d]. In 60% of cases, it was mild whereas, 40% cases showed intense expression (100%) located in the cytoplasm. A progressive increase in intensity was observed with grades of OED, i.e., intense expression in mild (65%), moderate (70%) and severe (80%). Similarly, WDSCC showed intense expression in 80% of cases while MDSCC and PDSCC both showed 85% and 95% intense expression, respectively.

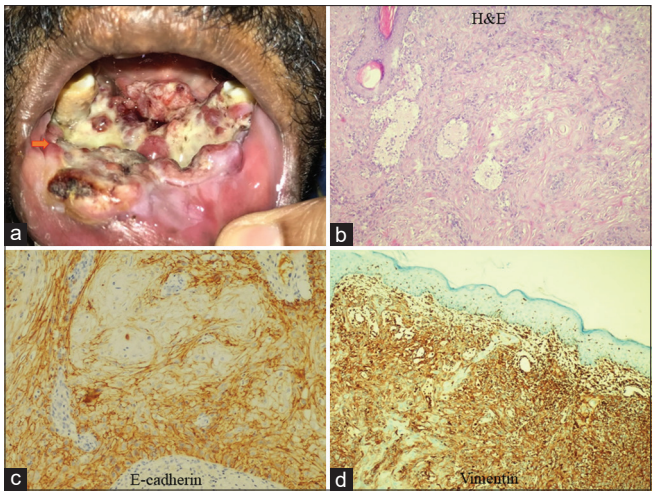

Figure 7: (a) Clinical picture of patient with ulcerative to exophytic growth involving the base of tongue, gingiva and lips; (b) Histopathology showing features of poorly differentiated squamous cell carcinoma underlying the normal adjacent epithelium (H&E, ×40); (c) E-cadherin showing mild patchy positivity with both membranous and cytoplasmic localization; (d) Vimentin showing strong intense cytoplasmic positivity in >50% of tumor cells (IHC, x20 & x40)

The expression of vimentin in the connective tissue was located in the cytoplasm of the cells among positive cases of the study group. Progressive increase in >50% expression was observed with grade of OED, i.e., mild (45%), moderate (60%) and severe OED (70%) which was also observed in OSCC group, i.e., WDSCC (80%), MDSCC (85%) and PDSCC (95%) [Figure 2d, 3d, 4d].

It was predominantly seen throughout in early (80%). A progressive throughout expression involving the entire connective tissue was observed with degree of OED, i.e., mild (60%), moderate (85%) and severe (95%). A throughout extent of expression was 80% (WDSCC), 85% each in MDSCC and PDSCC. These differences in the group were not significant (P>0.05).

DISCUSSION

Oral cancer is the 11th most common cancer in the world and ranks 6th globally in deaths. The incidence for oral cancer is the highest in India accounting approximately 30%–40% among all cancers.[2,9] A preinvasive stage of oral precancerous lesion always precedes OSCCs. Over 30 years, the malignant transformation of oral leucoplakia is 1%–20% while OSMF and oral lichen planus have malignant transformation of approximately 7%–13% and 0%–2%, respectively.[9-11]

The progression of these premalignant disorders to carcinoma is an multistage process and is preprogramed temporarily in the genome.[12] There are molecular studies that have explored a number of genes being amplified, altered, dysregulated in expression or deleted in head-and-neck tumorigenesis. The quench for early detection of the lesion and progression is necessary as it increases the chances of favorable treatment outcome and better prognosis.[2,12,13]

Dysplasia presence and severity in a potentially malignant oral lesion is currently the standard in predicting the risk for malignant transformation.[14] It has been observed that precancerous lesions with epithelial dysplasia progress to cancer more readily. The chances of malignant transformation in nondysplastic leucoplakia lack evidence currently considering the differences in the behavior or risk of malignant transformation.[15,16]

Malignant transformation in various carcinomas is associated with the loss of epithelial differentiation and a gain in a mesenchymal phenotype, which has been described in events associated with embryogenesis, healing and metastasis.[14,17] Epithelial to mesenchymal transition (EMT) may be a predictor of OSCC progression and prognosis as suggested in recent studies.[18,19] The concept of EMT was first proposed by Greenberg *et al*.[20] These features are noted in both OSCC progression and OED. These changes can be seen in the development of OSCC; the identification of genes or their products that play a role in the transition process may be the potential biomarkers of malignant transformation.[18,21]

E-cadherin is a calcium dependent cell surface adhesion molecule with 120-kDa, responsible for the control of cell motility in embryogenesis or tissue healing. Downregulation of e-cadherin allows migration of the regenerating epithelium over area of ulceration.[22,23] Hence, the role of e-cadherin in control of cell motility has led to the suggestion that it is an "invasion suppressor" molecule responsible for various carcinomas of head-and-neck region, prostrate, esophagus, stomach, pancreas and uterine cervix.[14,22-26] E-cadherin reduced expression has correlated with high proliferation, aggressive behavior, invasion, metastasis and poor prognosis of cancer.[2,27] Hence, it indicates the prognostic significance of e-cadherin during therapeutic intervention.

Vimentin is a type III intermediate filament protein of mesenchymal cells having a molecular weight 54 kDa. Vimentin was previously thought to be only a structural protein.[3,28] However, recent evidence suggests the role in regulation of various cellular functions including migration, cell signaling, cell attachment and tumor metastases.[29] Its expression correlates with poor prognosis in various carcinomas including OSCC.[3,29] The proposed location

of molecular markers specifically in the invasive fronts of tumor has more prognostic significance rather than their expression levels.[30] These reports together indicate that aberrant expression of vimentin is associated with tumor invasion and metastases. However, there are no reports available regarding its expression in precancerous lesions except for one who have demonstrated its expression in the hyperkeratotic lesions of oral mucosa.[31]

In the present study, we had focused in the identification of e-cadherin and vimentin markers role in early stages of oral tumor development and to further determine clinical significance of its expression for the prediction of high-risk oral lesions. E-cadherin expression in low-grade OED was similar to the control normal group of patients. A greater loss of e-cadherin expression with shift in localization to cytoplasmic was noted with increasing grades of OED and to OSCC. A shift from basal and parabasal intercellular expression of e-cadherin to the upper 1/3rd layers was noted in grades of OED, possibility due to delayed desquamation and immature cells. However, this parameter could not be applied to OSCC due to lack of the intact epithelium. Similar were the observations done by Yogesh *et al*.,[32] Kaur *et al*.,[33] Sridevi *et al*.,[2] Zhou *et al*.,[34] and Akhtar *et al*.[29]

Vimentin expression increased with the progression of OED and into OSCC. Myong had observed no immunoreactivity for vimentin in normal buccal mucosa.[35] Araujo *et al*. had a vimentin positivity in cells of histologically high grade of tumor and was associated with poor outcome on patient treatment.[36]

Myong[35] has reported a statistically significant inverse correlation between e-cadherin and vimentin immunoexpression, with $P < 0.001$ in their study on 119 cases, a finding concordant with our study. Carcinoma *in situ* lesions had 8.8% immunoreactivity, whereas microinvasive and invasive carcinomas were having higher vimentin expression in approximately 53.0% and 67.0% cases, respectively, findings concordant with our study.[37,38] Hence, a significantly increased vimentin expression can be a target for EMT marker in the progression of premalignant/carcinoma *in situ* to microinvasive or invasive squamous cell carcinoma.

CONCLUSION

With the limited number of cases, we were having the findings of variation in expression of e-cadherin and vimentin with various grades of OED and OSCCs in comparison to normal mucosa. Long-term follow-up will be required for patient's prognosis and prognosticate the malignant potential of premalignant lesions.

Abbreviations:

EMT: Epithelial mesenchymal transition, H&E: Hematoxylin and eosin, HRP: Histidine Rich protein, IHC: Immunohistochemistry, OED: Oral Epithelial Dysplasia, OSCC: Oral Squamous Cell Carcinoma, OSMF: Oral Sub Mucosal Fibrosis, WHO - World Health Organization.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. IEC approval Number-MCDRC/2017/10081.

Consent for publication

Written consent for publication and any additional related information was taken from the patient involved in the study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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