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Immunohistochemical expression of MUC1 in different grades of oral squamous cell carcinoma

K. C. Shobhita[1], N. D. V. N. Shyam[2], P. Preethi[3], R. Poornima[4], M. Priyanka[5], R. Shravani[6]

Department of Oral and Maxillofacial Pathology, Government Dental College and Hospital, Hyderabad, Telangana, India

ABSTRACT

Background: MUC1 mucin is a membrane-bound mucin encoded by the MUC1 gene. Alteration in glycosylation of mucins is associated with the development and progression of malignancy. Therefore, mucins are used as markers which are valuable to distinguish normal and diseased conditions. Although expression of MUC1 has been identified in a various neoplasms, only few studies have done to estimate MUC1 expression in oral carcinomas. **Aims and Objectives:** The aim of this study is to evaluate, compare, and correlate the MUC-1 immunoexpression and its significance in normal oral mucosa (NOM) and in different grades of oral squamous cell carcinoma (OSCC). **Materials and Methods:** Our study included 30 histopathologically diagnosed tissue sections of different grades of OSCC, which were immunostained for MUC1 mucin expression and 10 cases of NOM as controls. The percentage of the positivity of cells and staining intensity was analyzed according to the immunoreactive scoring system and statistically analyzed by Pearson's Chi-Square test. Membranous and cytoplasmic staining was considered as positive for MUC1 mucin immunoexpression. **Results:** There was statistically significant difference in the percentage of positivity of cells and staining intensity from NOM to OSCC and also in different grades of OSCC with a $P < 0.001$. **Conclusion:** The findings of our study suggest that MUC 1 plays an important role in pathogenesis and can be regarded as a valuable marker for OSCC.

Key words: Cell proliferation, differentiation, metastasis, MUC1 mucin, oral squamous cell carcinoma, tumorigenicity

INTRODUCTION

A pressing problem in the world is oral cancer, and the WHO predicts a worldwide continuous increase in the number of oral cancer patients. Regardless of the easy access of oral cavity for clinical examination, oral squamous cell carcinoma (OSCC) is routinely diagnosed in advanced stages. Most common reasons are the initial wrong diagnosis and the ignorance from the patient or from attending physician. Early detection of disease progression remains a challenging task mainly due to lack of a reliable molecular marker that predicts both early diagnosis and prognosis of this devastating disease.[1,2]

Mucins are glycoproteins with high molecular weight that plays a vital role in cell growth, differentiation, and cell signaling. MUC1 mucin is a membrane-bound mucin encoded by the MUC1 gene.[2,3] MUC-1 promotes neoplastic transformation, tumor survival, angiogenesis, and metastasis.[4]

The present study was conducted to evaluate, compare, and correlate the expression of MUC1 mucin protein and its significance in normal oral mucosa (NOM) and OSCC by Immunohistochemical method.

MATERIALS AND METHODS

The present study was conducted on the paraffin-embedded blocks retrieved from the archives of the Department of Oral and Maxillofacial Pathology, Government Dental College and Hospital, Hyderabad, Telangana. A total of 40 cases which were clinically and histopathologically diagnosed as NOM (n = 10) and OSCC (n = 30; WDOSCC=10, MDOSCC=10, PDOSCC=10) were stained for MUC1 mucin.

Exclusion Criteria

The following criteria were excluded from the study:

1. Patients with previous history of malignancy.
2. Patients undergoing treatment for malignancy (surgery, chemotherapy, and radiotherapy) and,
3. Patients with metastatic tumors in jaws from systemic malignancies.

The percentages of positive cells are evaluated. An additional tissue section was taken from all the cases and stained with hematoxylin and eosin for comparative purpose.

Immunohistochemistry

3 um thick sections were extracted from selected tissue blocks and loaded on to silane-coated slides. Following deparaffinization by heating on a slide warmer for 1 h at 60°C and treatment with xylene, sections were rehydrated in ethanol and water. Then, sections were placed in a commercial microwave antigen retrieval system (EZ Retrieval System, Pathnsitu Biotechnologies Pvt., Ltd) containing tris (hydroxymethyl) aminomethane ethylene-diamine-tetraacetic acid buffer and treated at 95°C for five cycles: 5 min for

Address for correspondence:
Kandikatla Carmel Shobhita, Room no: 332, Department of Oral and Maxillofacial Pathology, Government Dental College and Hospital, Hyderabad, Telangana, India. Phone: +91-9989298550. E-mail: dolly.kandikatla1990@gmail.com

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first cycle and 3 min each for remaining cycles for antigen retrieval. Antigen-retrieved sections were allowed to cool for 30 min and then rinsed in distilled water followed by washing in TRIS buffer. Further slides were treated with 3% hydrogen peroxide for 5 min to block endogenous peroxidase activity. Then, tissue sections were incubated with a prediluted primary antibody against MUC1 (Rabbit Monoclonal, Clone EP 85, Pathnsitu Biotechnologies Pvt., Ltd.) for 30 min at room temperature. Then, tissue sections were incubated with horseradish peroxidase one-step polymer - secondary antibody. Immunoreactions for MUC1 were visualized with diaminobenzidine chromogen. Finally, sections were counterstained with Harris hematoxylin, dehyrated in ethanol and xylene, and finally mounted with dibutyl phthalate xylene.

Interpretation of the Slides

The staining pattern in colon carcinoma was used as the positive control. The distribution of positive cells was first examined under low magnification (×10); among which, 5 fields are randomly selected to calculate the percentage of positive cells under high magnification (×40) and we also observed staining intensity of MUC1 and grading was done according to the immunoreactive score (IRS) given by Remmele and Stegner as shown in the Table 1.[5] Membranous and cytoplasmic staining was considered as positive for MUC1 mucin immunoexpression. All IHC-stained slides together with the corresponding H and E sections were analyzed by two observers.

Statistical Analysis

The obtained data were subjected to SPSS software version 20.0. Pearson's Chi-square test was computed to calculate the association between the type of condition and percentage of positivity of cells.

RESULTS

Analysis revealed that majority of the samples in control group showed no positive cells. However, well-differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma, and poorly differentiated squamous cell carcinoma (PDSCC) groups showed that majority of the samples showed 51–80% and >80% positivity of cells. There was a shift in the percentage positivity of cells from controls to OSCC groups. This association of greater percentage of positivity of cells with squamous cell carcinoma samples and association of no positive cells with NOM were found to be statistically significant with a $P < 0.01$ [Table 2 and Graph 1].

There was also statistically significant difference in the intensity of staining in NOM and different grades of OSCC with $P< 0.01$ [Table 3 and Graph 2].

When IRS score was compared, there was statistically significant difference between NOM and different grades of OSCC ($P < 0.01$) [Table 4 and Graph 3].

Table 1: IRS

A (percentage of positive cells)	B (intensity of staining)	IRS score (multiplication of A and B)
0 = no positive cells	0 = no color reaction	0–1 = negative
1≤10% of positive cells	1 = mild reaction	2–3 = mild
2 = 10–50% positive cells	2 = moderate reaction	4–8 = moderate
3 = 51–80% positive cells	3 = intense reaction	9–12 = strongly positive
4≥80% positive cells	Final IRS score (A × B): 0–12	

IRS: Immunoreactive score

Table 2: Percentage of positive cells in different groups

Group	Positive cells					Total	*P* value
	No positive cells	<10% positive cells	10–50% positive cells	51–80% positive cells	>80% positive cells		
NOM	8 (80.0)	1 (10.0)	1 (10.0)	0 (0.0)	0 (0.0)	10 (100.0)	$X^2 = 63.375$ $P<0.01$
WDSCC	0 (0.0)	0 (0.0)	4 (40.0)	4 (40.0)	2 (20.0)	10 (100.0)	
MDSCC	0 (0.0)	0 (0.0)	1 (10.0)	6 (60.0)	3 (30.0)	10 (100.0)	
PDSCC	0 (0.0)	0 (0.0)	1 (10.0)	4 (40.0)	5 (50.0)	10 (100.0)	
Total	9 (18.0)	3 (6.0)	11 (22.0)	16 (32.0)	11 (22.0)	50 (100.0)	

NOM: Normal oral mucosa, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

Table 3: Staining intensity among different groups

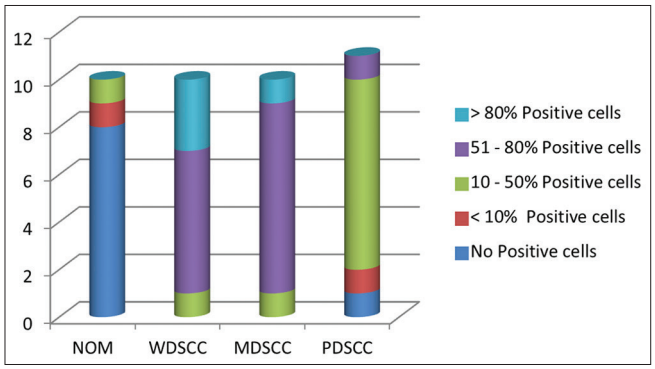
Group	Intensity of staining			Total	Chi-square
	Negative	Mild staining	Moderate staining		
NOM	8 (80.0)	2 (20.0)	0 (0.0)	10 (100.0)	$X2=35.667$ $P<0.01$
WDSCC	0 (0.0)	4 (40.0)	6 (60.0)	10 (100.0)	
MDSCC	0 (0.0)	5 (50.0)	5 (50.0)	10 (100.0)	
PDSCC	1 (10.0)	6 (60.0)	3 (30.0)	10 (100.0)	
Total	10 (20.0)	25 (50.0)	15 (30.0)	50 (100.0)	

NOM: Normal oral mucosa, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

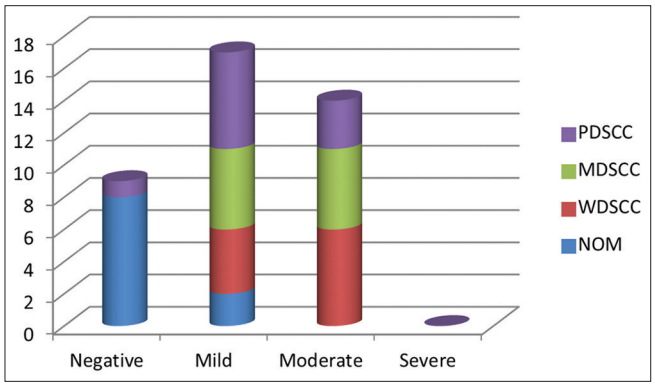
Table 4: Association of type of lesion with IRS grading

Group	IRS score			Total	Chi-square
	Negative expression	Positive, weak expression	Positive, intermediate expression		
NOM	8 (80.0)	2 (20.0)	0 (0.0)	10 (100.0)	X^2=33.208 *P*<0.01
WDSCC	0 (0.0)	4 (40.0)	6 (60.0)	10 (100.0)	
MDSCC	0 (0.0)	5 (50.0)	5 (50.0)	10 (100.0)	
PDSCC	1 (10.0)	6 (60.0)	3 (30.0)	10 (100.0)	
Total	10 (20.0)	24 (48.0)	16 (32.0)	50 (100.0)	

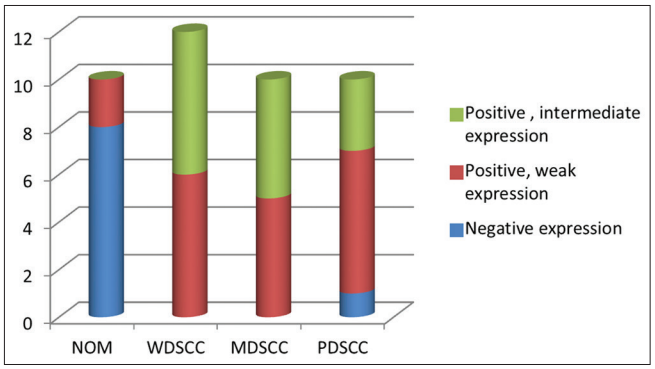
NOM: Normal oral mucosa, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma



Graph 1: Percentage of positive cells in different groups



Graph 2: Staining intensity among different groups



Graph 3: Immunoreactive score grading in different groups

DISCUSSION

The second most leading cause of mortality in economically developed countries (next to heart diseases) and the third most leading cause of death in developing countries (next to heart diseases and diarrheal diseases) are cancer.[6] Among all cancers, oral cancer is the most prevalent in the world, and it ranks third among cancers in South and Central Asia. In males, it is the most common cancer, while in females, it is third most common cancer in India.

OSCC constitutes 95%–98% of all oral cancers.[6] It is the most common cancer accounting for 12% of all cancers in men and 8% of all cancers in women.[3] In India, one of the major factors which worsen the disease prognosis is late diagnosis of carcinoma.[7] It is known for its detrimental and lethal effect.[8] The survival rates of OSCC were 59.9% in 1 year, 40.7% in 2 years, and 27.8% in 5 years.[9]

Knockdown and overexpression studies of MUC1 discovered that it leads to increased anti-adhesive properties and tumorigenicity in number of systems. Under in vitro conditions, by mediating binding to some molecular ligands and blocking binding with other ligands, overexpression of MUC1 has been shown to reduce adhesion between neighboring cells and between cells and extracellular matrix.[10]

Mucins are multifunctional glycoproteins that are thought to exclusively represent the principal component of mucus, which help in protecting and lubricating epithelial surfaces within the human body. In addition, mucins are also involved in signaling pathways that direct coordinated cellular responses such as secretion of specialized cellular products, cell proliferation, differentiation, and apoptosis.[11]

It is accepted generally that the structure and distribution of cell surface glycoconjugates change during malignant transformation and tumor progression. The findings of the present study suggest that MUC1 mucin may be a useful indicator of malignant potential given its increased rate of expression during disease progression to OSCC. This MUC1 upregulation may reflect early cellular changes from normal cell-cell and cell-matrix interaction toward bizarre, pathophysiologic, heterotypic cell surface adhesion properties. Expression of MUC1 mucin may be associated to the invasion or metastasis of carcinoma cells.[9-11]

Cancer cells express aberrant forms or amounts of mucins. These aberrant forms arise as a result of the deregulation of mucin core proteins and the enzymes that modify them, during the transformation of tumor cells.[3,12]

Mucins are used by cancer cells for protection from adverse growth conditions and to control the local molecular microenvironment during invasion and metastasis.[11]

It is well established that, in several neoplasms, membrane-associated mucin MUC1 is aberrantly glycosylated and overexpressed. The cytoplasmic tail of MUC1 can bind and signal through beta-catenin and the mitogen-activated protein kinase. The early studies showed that MUC1 was phosphorylated on both serine and tyrosine residues within the cytoplasmic tail and also changes in phosphorylation of cytoplasmic MUC1 correlate with differences in cell adhesion.[10]

Many studies have indicated that MUC1 mucin can act as an antiadhesion molecule.[11] Overexpression of MUC1 mucin on the cell surface reduces cell-cell and cell-extracellular matrix adhesion, perhaps the large, elongated, and rigid structure of MUC1 mucin interferes with interactions between adhesion molecules and their ligands.[13] Cells that express abundant MUC1 mucin have decelerated levels of interaction between integrins and the extracellular matrix.[14] MUC1 mucin overexpression was associated with invasive and metastatic tumors of the pancreas, gallbladder, colon, and oral epithelium.[11] In cancer cells, increased expression of MUC1 promotes invasion of cancer cell through beta-catenin, resulting in the initiation of epithelial-mesenchymal transition which promote the formation of metastasis.[15,16]

In the present study, cases of different grades of OSCC and well-differentiated OSCC showed membranous and cytoplasmic staining positive staining, also in keratin pearls [Figure 1], which is in accordance with Kumar *et al*. In moderately differentiated OSCC, membranous moderate staining was observed [Figure 2], while in poorly differentiated OSCC, only membranous staining was observed [Figure 3]. There is an overexpression of MUC1 in OSCC compared with its normal and counterpart, as seen in a study conducted by Kumar *et al.,*[3] Nitta *et al.,*[13] Narashiman *et al.,*[17] and Kaur *et al.*[10]

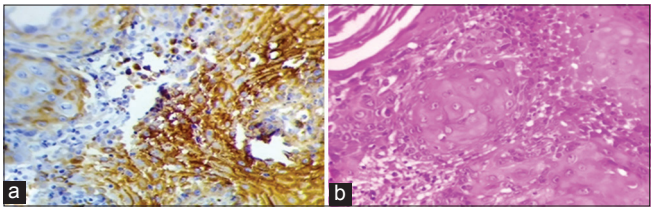


Figure 1: (a) Photomicrograph of the section shows membrane and cytoplasmic staining in the epithelium and keratin pearl of well-differentiated squamous cell carcinoma (IHC stain, ×40). (b) The corresponding H and E section, ×40

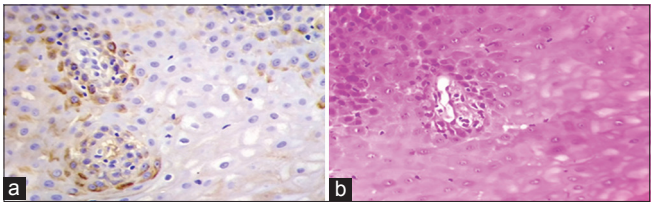


Figure 2: (a) Photomicrograph of the section shows membranous and cytoplasmic staining in the epithelium of moderately differentiated squamous cell carcinoma (IHC stain, ×40). (b) The corresponding H and E section, ×40

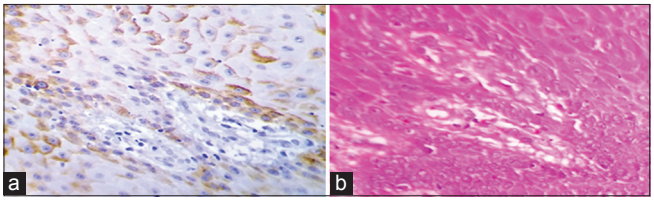


Figure 3: (a) Photomicrograph of the section shows membranous staining in the epithelium of poorly differentiated squamous cell carcinoma (IHC stain, ×40). (b) The corresponding H and E section, ×40

The association of greater percentage positivity of cells with OSCC samples than NOM was found to be statistically significant with $P < 0.01$. This might be because the cancer cells utilize mucins for cell proliferation. The association of more staining with OSCC samples in comparison with NOM was found to be significant statistically with $P < 0.01$. This might be because the cancer cells use mucins for their survival, protection from innate immunity, and invasion which are the characteristic feature of malignancy.[10-12]

There is a progressive increase of positive expression from NOM to OSCC which was found statistically significant with a $P < 0.01$, as IRS values are based on the percentage of positivity and staining intensity. The histological grades of OSCC were also compared, and we found a significant decrease in the immunoexpression of MUC1 from well-differentiated to poorly differentiated OSCC through moderately differentiated OSCC, as seen in Narashiman *et a*l.,[17] Weed *et al.,*[18] and Guillem *et al.*[19] This is attributed to the inability of the less differentiated squamous cells to express mucins compared with that of well-differentiated cells of OSCC. This may be comparable to an unexplained complex immunoreactive phenomenon. Probably this might be due to the fact that the decelerated catabolism of certain inhibitory proteins for MUC1 immunoexpression in well-differentiated mature atypical cells. In PDSCC, there might be increased synthesis of certain intrinsic inhibitory proteins for MUC1 for some unknown mechanism which altered maturation and de-differentiated of cancer cells.[17-19]

In general, MUC1 is well expressed in the well-keratinized areas which are usually seen in well-differentiated OSCC. Tumor cells produce MUC1 mucin which is released into the circulation and captured by IgG antibodies forming MUC1-IgG immunocomplexes.[20]

Limitations

1. Our study included smaller sample size. Future studies with large sample size may give better results.
2. Our study did not include oral potentially malignant disorders (OPMD). Future studies with including OPMD may give better results as most of the OSCCs are preceded by OPMDs.

CONCLUSION

The present study concludes that upregulation of MUC1 mucin expression in malignant lesions might play a vital role in the pathogenesis and its progression. It can also be a useful marker for prediction of the metastatic/invasive potential of OSCC. Hence, MUC1 mucin can be regarded as a valuable marker for OSCC. Future studies on comparative analysis of mutant types of MUC1 and its variable expression in invasive and non-invasive squamous cell carcinomas should be done.

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