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Pathology

TO EVALUATE THE EXPRESSION OF MUC1 IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT **Background:** Mucins alteration in glycosylation is associated with the development and progression of malignant diseases. Therefore, mucins are used as valuable markers to distinguish normal and disease conditions. Many studies on MUC1 expression have been conducted on variety of neoplastic lesions other than head and neck region. None of the study has made an attempt to show its significance in potentially malignant disorders (PMDs) and oral squamous cell carcinoma (OSCC). Hence, ours is one of the pioneer studies done to assess and evaluate the same. This study aims to evaluate the expression of MUC1 in oral squamous cell carcinoma. **Materials and Methods:** In this cross-sectional study total 50 patients with archival retrieved, formalin fixed, paraffin embedded, histologically diagnosed case of different grades of oral squamous cell carcinoma tissues, Immunohistochemistry (IHC) for MUC1 was performed, and the overall percentage of positive cells along with distribution and localization of immune-expression was studied. **Results:** There was statistically significant difference in the percentage of positivity of cells and staining intensity of muc-1 and also in different grades of OSCC and the association of type of lesion with IRS grading was also found significant($p<0.001$). The association of greater percentage of positivity of cells with squamous cell carcinoma samples and association of number of positive cells with normal oral mucosa is found to be statistically significant($p<0.001$). **Conclusion:** We conclude that MUC 1 Mucin biomarker can be used to detect higher grades of oral squamous cell carcinoma and hence, early detection in preventing invasion and metastasis.

KEYWORDS : MUC1 Mucin, Normal Oral Mucosa, Oral Squamous Cell Carcinoma, Oral Submucous Fibrosis

INTRODUCTION

Oral cancer ranks from sixth to eighth most common cancer worldwide, with a great variability in incidence among countries. In South Asia, over 90.0% of oral malignancies are known to arise from pre-existing potentially malignant disorders (PMD's) such as leukoplakia, erythroplakia and oral submucous fibrosis (OSF). Early detection of disease progression remains a challenging task mainly due to lack of adequate early prognostic markers.[2]

Tumor markers should be highly specific for any tumor type and also should be highly sensitive to avoid false positive results. Though tumor markers are not regarded reliable for diagnosis as they are an adjunct to routine histopathology technique with H & E stains. There is an increasing number of tumor markers for oral squamous cell carcinoma every year. Recent studies have reported on MUC – 1 on changes from normal to oral squamous cell carcinoma and hence considered as useful indicator of the disease process. MUC–1 overexpression in metastatic tumors has been reported and is correlated with aggressiveness, poor response to therapy and poor survival.

As reported earlier, around 95.0-98.0% of all oral malignancies seem to be oral squamous cell carcinoma and delayed diagnosis seem to be the reason for poor prognosis of the disease. The high percentage of the disease are due to various etiological factors like chewing and smoking tobacco, alcohol intake and viral infections leading to development of premalignant lesions, invasive oral squamous cell carcinoma followed by metastasis. Though recent advances have evolved in diagnosing and treatment techniques of OSCC, yet the disease presents with poor prognosis and decreased survival rate if detected in the later stages of the disease.

Great advances have been achieved in general patient care, surgical techniques, as well as local and systemic adjuvant therapies, while the mortality rate of OSCC is still high and It is known for its detrimental and lethal effect. The survival rates of OSCC were 59.9% in 1 year, 40.7% in 2 years, and 27.8% in 5 years.[7.] Most common reason 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.

Mucins are high molecular weight glycoproteins that play a major role in cell growth, differentiation and cell signalling. Mucin gene expression is highest in the respiratory, digestive and reproductive systems. The cancer cells use mucin for cell proliferation, survival, invasion, metastatic growth and protection against innate immunity. An aberrant expression of MUC1 in various human cancers has highlighted its role in the pathogenesis of cancer.[1]

MUC–1 mucin has been reported as an anti-adhesion molecule by many studies and overexpression on the cell surfaces reduces adhesion between cell to cell and extra cellular matrix indicating non interactions between the integrins and extra cellular matrix. Hence, in invasive and metastatic carcinomas there is an overexpression of MUC–1 mucin has been reported. Immuno-expression of MUC–1 expression has been reported to significantly decrease from well differentiated to poorly differentiated OSCC which may be due to the lack of expression of mucins in the less differentiated squamous cells.[5] Within this background the present study has been undertaken, to evaluate the expression of MUC-1 in oral squamous cell carcinoma.

MATERIALAND METHODS

This cross-sectional study was performed over 50 oral cavity samples to analyse Immunohistochemical expression of MUC1 Mucin was done on the archival retrieved, formalin fixed, paraffin embedded, histologically diagnosed case of different grades of oral squamous cell carcinoma tissues, obtained from the Department of Pathology, S.N. Medical College, Agra over a period of one and a half year (from October 2019 to March 2021). The research procedure followed was in accordance with the approved ethical standards of Department of Pathology, S.N. Medical College, Agra.

Study Sample: Biopsy of oral cavity samples

Inclusion Criteria

- Histopathologically confirmed cases of oral SCC
- Tissue should be adequate for further processing for MUC1
- Clinical details should be available for correlation.

Exclusion Criteria

- Patients with metastatic tumours in jaws from systemic malignancies.
- Inadequate and autolysed tissue.
- Individuals with traumatic ulcers and herpetic lesions.

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

IHC Methodology

Formalin fixed paraffin embedded (FFPE) tissue blocks of biopsy specimens, diagnosed as oral squamous cell carcinoma (OSCC) were taken. From each FFPE tissue block,3-4 μ thick sections were cut and

stained by H&E stain for histopathological grading. Immunohistochemical study was carried out using polymer-labelling technique (Dako, Envision). Sections were dewaxed, washed in alcohol and antigen retrieval was carried out with 10mM Citra solution at 125°c for 30 seconds followed by 90°c for 10 seconds. Slides were cooled naturally and bought to room temperature. 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 (MUC1) for 60 minutes. Sections were again washed with PBS, incubated with polymer for 30 minutes, and washed again with PBS.

Analysis of Immunoreactivity for MUC-1

The distribution of positive cells was first examined under low magnification (x10); among which, 5 fields are randomly selected to calculate the percentage of positive cells under high magnification (x40) and we also observed staining intensity of MUC 1 and grading was done according to the immunoreactive score (IRS) given by Remmele and Stegner. Membranous and cytoplasmic staining was considered as positive for MUC1 mucin immune-expression. All IHC-stained slides together with the corresponding H&E sections were analysed by the observer.

Scores And Inference for Area of Staining [A]

0 - No positive cells
1 - ≤10% of positive cells
2 – 10-50% of positive cells
3 – 51-80% of positive cells
4 - ≥80% positive cells

Scores And Inference for Intensity of Staining [B]

0 - No colour reaction
1 - Mild
2 - Moderate
3 – Intense

IRS Score [A×B]

0 – 1 = Negative
2 – 3 = mild
4 – 8 = moderate
9 – 12 = strongly positive
Immunoreactivity Score (IRS) = multiplication of A & B.
[A=percentage of positive cells, B = intensity of staining].

Statistical Analysis

Data was analysed using Statistical Package for Social Sciences, version 23 (SPSS Inc., Chicago, IL). Discrete (categorical) data were summarized as in proportions and percentages (%) while quantitative data were summarized as mean (SD), median or mode. Chi square and other appropriate tests were used to check associations. P<0.05 was considered significant.

OBSERVATIONS/RESULTS

In the present study the age of presentation of OSCC ranged from 26 to 81 years, with the mean age of 47.7 years. Gender distribution was 85.0% men and 15.0% women (Table 1). Out of 40 OSCC cases 37.5% were WDSCC, 37.5% MDSCC and 25.0% were PDSCC (Table 2). In our study the majority of the studied patients were having tumour in tongue (35.0%) followed by buccal mucosa (17.5%) and tonsillar area (5.0%) (Figure 1). In our study, the association of greater percentage positivity of cells with OSCC samples than NOM was found to be statistically significant (p<0.01) (Table 2). We observed statistically significant difference in the intensity of staining in NOM and different grades of OSCC (p<0.001) (Table 3). In the present study on comparing IRS score there is statistically significant difference between NOM and different grades of OSCC (p<0.001) (Table 4)

Table 1: Distribution of the Studied Patients on the Basis of their Demographic Details

Parameters	No. of cases (n=40)	Percentage
0-10 years	00	0.0%
11-20 years	00	0.0%
21-30 years	05	12.5%
31-40 years	06	15.0%
41-50 years	15	37.5%
51-60 years	08	20.0%
61-70 years	04	10.0%
71-80 years	01	2.5%
81-90 years	01	2.5%
Mean Age (years)	47.7±	
Gender Male	34	85.0%
Gender Female	06	15.0%

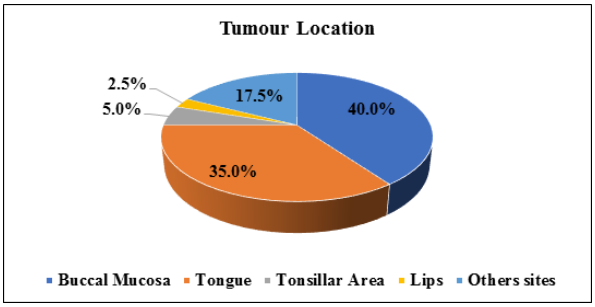

Figure 1: Distribution of OSCC Cases (n=40) on the Basis of Site of oral Cavity

Table 2: Comparison of Percentage of Positive Cells in Different Groups

Groups	Positive Cells					Total	p-value
	No positive cells	<10.0 %	10.0-50.0 %	51.0-80.0 %	>80.0 %		
Benign	7	2	1	0	0	10	<0.001
MDSCC	0	0	4	9	2	15	
PDSCC	0	0	1	5	4	10	
WDSCC	0	0	7	4	4	15	
Total	7	2	13	18	10	50	

Table 3: Comparison of Staining Intensity of MUC-1 with Different Groups

Groups	Intensity of staining				Total	p-value
	Negative	Mild staining	Moderate staining	Intense		
Benign	7	3	0	0	10	<0.001
MDSCC	0	4	10	1	15	
PDSCC	0	5	4	1	10	
WDSCC	0	6	7	2	15	
Total	7	18	21	4	50	

Table 4: Association of Type of Lesion with IRS Grading

Groups	IRS Score				Total	p-value
	Negative (0-1)	Mild (2-3)	Moderate (4-8)	Strong (9-12)		
Benign	7	3	0	0	10	<0.001
MDSCC	0	4	10	1	15	
PDSCC	0	5	4	1	10	
WDSCC	0	6	7	2	15	
Total	7	18	21	4	50	

DISCUSSION

Of the top 10 cancers reported in the globe, Oral Squamous cell carcinoma is being highly prevalent and reported in all countries, though there may be geographical variations in incidence. Oral Squamous cell carcinoma is being frequently reported in Central and South Asian countries related to the use of tobacco products and alcohol and India has been reported with 50.0% of mortality as on 2018 due to being diagnosed at later stages. Immunoexpression of Tumour markers has become a boon to early detection and hence preventing early invasion and metastasis.

Mucins play an important role in cell growth, cell differentiation and cell signalling.[5] They belong to a family of high molecular weight glycoproteins. Most of the mucins are membrane bound due to the presence of a hydrophobic membrane that allows retention in the plasma membrane. Mucins are basically secreted as principal component of mucus. Sometimes, they are secreted as a component of saliva. Till now, about 20 different types of human mucin genes have been identified. Among them, MUC1 Mucin is a transmembrane mucin like glycoprotein encoded by the MUC1 gene and its expression has been identified in a variety of malignancies, including malignant oral lesions.

OSCC constitutes 95.0-98.0% of oral cancers. In India, one of the major factors which worsen the disease prognosis is late diagnosis of

carcinoma.It is known for its detrimental and lethal effect.The survival rates of OSCC were 59.9% in 1 year,40.7% in 2 years and 27.8% in 5 years.[8]

In the present study the age of presentation of OSCC ranged from 26 to 81 years, with the mean age of 47.7 years. Gender distribution was 85.0% men and 15.0% women. Out of 40 OSCC cases 37.5% were WDSCC, 37.5% MDSCC and 25.0% were PDSCC. Our findings were in accordance with Kumar MH et al[1] who reported that the age presentation of OSCC ranged from 27 to 76 years, with the mean age of 45.8 years. Gender distribution was 45.0% men and 55.0% women. Out of twenty OSCC cases 35.0% showed poorly differentiated OSCC and 65.0% showed well-differentiated OSCC. Thakur A et al reported that of 30 cases of OSCC, of which 26 were well-differentiated OSCC (WDOSCC), 3 were moderately differentiated OSCC and 1 was poorly differentiated OSCC. The study group comprised 23 males and 7 females with age ranged from 24 to 75 years with a mean of 49.4 years. Most of the patients affected belonged to 40 to 60 years of age group. Guerrero J C H et al 58.4% were men, giving a male: female ratio of 1.4:1, and the mean age was 62.5±14.9 years.

In our study the majority of the studied patients were having tumour in tongue (35.0%) followed by buccal mucosa (17.5%) and tonsillar area (5.0%). The findings of our study were consistent with Abdulla R et al who reported that the most common site of involvement among the young was tongue and buccal mucosa respectively which is in concordance with our study. However, our study was not in concordance with Rathee R et al who stated Alveolar ridge (37.5%) as the most frequent site of involvement followed by buccal mucosa (29.2%) reporting a non-significant difference ($p>0.05$) correlating site with different grades of oral squamous cell carcinoma.

In our study, the association of greater percentage positivity of cells with OSCC samples than NOM was found to be statistically significant ($p<0.01$). These findings are similar to that of the results obtained by Kumar MH et al.[1] They also observed MUC1 Mucin immunoreactivity in 2 out of 20 cases of NOM. NOM showed weak staining as Mucins are involved in signalling pathways, cell differentiation, cell proliferation and apoptosis. But these findings are in contrast to that of the studies conducted by Nitta T et al[12] and Thakur A et al.[14] Their studies did not reveal any immune-reactivity to MUC1 Mucin in control group samples that is normal oral mucosa. This might be because the cancer cells utilize mucins for cell proliferation.

We observed statistically significant difference in the intensity of staining in NOM and different grades of OSCC ($p<0.001$).

In a study by Thakur A et al[14], out of 30 cases, 9 were negative and 21 were positive for immune-expression of MUC1. The distribution pattern MUC1 immuno-positive cells in 21 OSCC cases were diffuse in 11 cases and focal in 10 cases. This distribution pattern is in accordance with the study by Nitta T et al[12] and Kumar MH et al.[1] They also reported focal and diffuse pattern of immunostaining in their respective studies. Out of 21 cases, 30.0% cases showed mild intensity, 23.3% cases showed strong intensity and 16.7% cases showed moderate intensity. The moderate-to-strong cytoplasmic reactivity of MUC1 may be due to the intracellular transport of the DF3 antigen. Membrane glycoproteins are synthesized in the rough endoplasmic reticulum and transported to the Golgi complex.[12] According to Shobitha KC et al[5] there was also statistically significant difference in the intensity of staining in NOM and different grades of OSCC with $P<0.01$ depicted the similar results. 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.

In the present study on comparing IRS score there is statistically significant difference between NOM and different grades of OSCC ($p<0.001$). Our findings were comparable to the findings of Shobhita KC et al[5] who reported that when IRS score was compared, there was statistically significant difference between NOM and different grades of OSCC.

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 immune-expression of MUC1 from well-differentiated to poorly differentiated OSCC through moderately differentiated OSCC, as seen in Narashiman S et al, Weed DT et al and Guillem P et al. This is attributed to the inability of the less differentiated squamous cells to express mucins compared with that of well-differentiated cells of OSCC.

Studies have shown that MUC1 overexpression leads to loss of cell polarity and MUC1 acts as an anti-adhesion molecule. Overexpression of MUC1 on the cell surface reduces cell-cell and cell-extracellular matrix adhesion, probably because the large, elongated and rigid structure of MUC1 interferes with interactions between adhesion molecules and their ligands.[12]

Limitations of the Study

- Our study included smaller sample size. Future studies with large sample size may give better results.
- 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

From our study, we have observed that MUC-1 mucin is well expressed in the well keratinized areas, which are often seen in well differentiated OSCC. From our study, we would like to conclude that upregulation of MUC-1 Mucin immuno-expression in neoplastic lesions may play a pivotal role in the pathogenesis and progression of these lesions. MUC-1 Mucin may also serve as a useful marker for the prediction of metastatic potential, invasive potential and prognosis of OSCC. Hence, MUC-1 Mucin can be regarded as an important prognostic marker for OSCC

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