Expression of MUC1 mucin in potentially malignant disorders, oral squamous cell carcinoma and normal oral mucosa: An immunohistochemical study

M Harish Kumar, Karpagaselvi Sanjai, Jayalakshmi Kumarswamy, Roopavathi Keshavaiah, Lokesh Papaiah, S Divya

Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research Centre, Bengaluru, Karnataka, India

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% of oral malignancies are known to arise from preexisting potentially malignant disorders (PMDs) such as leukoplakia, erythroplakia and oral submucous fibrosis.
fibrosis (OSF). Early detection of disease progression remains a challenging task mainly due to lack of adequate early prognostic markers.[1]-[4]

Mucins are high molecular weight glycoproteins that play a major role in cell growth, differentiation and cell signaling. Mucin gene expression is highest in the respiratory, digestive and reproductive systems.[5]-[9] The cancer cells use mucin for cell proliferation, survival, invasion, metastatic growth and protection against innate immunity.[6,7,10] An aberrant expression of MUC1 in various human cancers has highlighted its role in the pathogenesis of cancer.[5,7,8,11] This study was conducted to evaluate and compare the expression of MUC1 and its significance in normal oral mucosa (NOM), oral squamous cell carcinoma (OSCC) and PMD’s.

MATERIALS AND METHODS

The study was conducted on the paraffin-embedded blocks retrieved from the archived files of Department of Oral Pathology and Microbiology. A total of sixty cases which were clinically and histopathologically diagnosed as OSCC (n = 20; well-differentiated = 13 and poorly differentiated = 7), PMD’s (n = 20, epithelial dysplasia = 10 and OSF = 10) and NOM (n = 20) were stained for MUC1 mucin.

Immunohistochemical detection of MUC1 mucin

Tissues of 3.5 μm were cut and transferred to 3-amino-propyl-triethoxy-silane coated slides and incubated overnight at room temperature. Antigen retrieval of sections immersed in citrate buffer solution was done using a pressure cooker. Endogenous peroxidases were blocked (Novacastra, Leica Systems, UK) at room temperature for 15 min. Then sections were incubated with primary anti-MUC1 mucin monoclonal antibody (Thermo Scientific Pvt. Ltd., USA) for 1 h followed by incubation with biotinylated secondary antibody (Novacastra, Leica Systems, UK) for 30 min. Then a drop of streptavidin was added from secondary antibody kit (Novacastra, Leica Systems, UK) for 30 min followed by incubation with 3′diaminobenzidine-tetrahydrochloride for 5–10 min. Then the sections were counterstained with hematoxylin and mounted. Carcinoma of breast tissue [Figure 1] was used as positive control and for negative controls TRIS buffered saline replaced the primary antibody.

Interpretation of the slides

The stained sections were scanned under low power to determine the area that stained brown color and was considered as positive for MUC1 mucin expression. Cytoplasmic and membranous staining were considered as positive immunoreaction for MUC1 mucin.[12,13]

In a randomly selected five fields, 100 cells were considered in each field. Out of 100 cells MUC1 mucin positively stained cells were counted. Two observers evaluated all the slides.

RESULTS

In NOM, 2 out of 20 cases (0.75%) MUC1 mucin immunoreactivity was observed [Figure 2]; all the 20 cases of OSCC (44%) expressed immunoreactivity for MUC1 [Table 1 and Graph 1].

Of the twenty specimens of PMD’S, (28%) 10 of oral epithelial dysplasia exhibited membranous staining in the basal, parabasal and spinous layer cells [Figures 3 and 4]. Of 10 cases of OSF, 9 cases showed immunoreactivity in the basal, parabasal and spinous layer cells [Figure 5] and one case did not show any positivity [Table 2]. Among twenty specimens of OSCC, 13 of well-differentiated OSCC and seven of poorly differentiated OSCC showed both cytoplasmic and cell membrane staining and the distribution pattern was focal or patchy. In well-differentiated OSCC, the keratin pearls also showed immunoreactivity. Higher mean immunohistochemical score was observed in OSCC followed by PMD’S and NOM. The difference in immunohistochemical score among the groups was found to be statistically significant (P < 0.001).

Statistically significant difference in mean immunohistochemical score was observed between OSCC and PMD’S (P < 0.01), OSCC and NOM group (P < 0.001) as well as between PMD’S group and NOM group (P < 0.001). However, no significant difference in immunohistochemical score was observed between poorly differentiated OSCC and well-differentiated OSCC groups (P < 0.301) [Table 3 and Graph 2].
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DISCUSSION

In India, OSCC is the most common cancer accounting for 12% of all cancers in men and 8% of all cancers in women.\textsuperscript{[14]} In the oral cavity; OSCC is the most prevalent malignant neoplasm.

PMD’s is defined by WHO 2005 as “the risk of malignancy being present in a lesion or condition either at time of initial diagnosis or a future date.”\textsuperscript{[15]} Leukoplakia is defined as “a white plaque of questionable risk having excluded other known diseases or disorders that carry no increased risk of cancer.” Multiple studies over the years have shown a malignant transformation rate of 3.6–17.5%.\textsuperscript{[1]} OSF is a chronic debilitating disease of oral cavity associated with arecanut (betel nut) chewing, affecting all parts of oral mucosa and oronasopharynx. OSF has a malignant transformation rate of about 0.5–6%.\textsuperscript{[15]}

In recent years, numerous prognostic factors associated with OSCC have been identified, some of them are inherent to the patient and others associated with the genetic profile of the malignant epithelial cells which reflect tumor aggressiveness.\textsuperscript{[16]}

Mucins are heavily glycosylated proteins that act as a molecular barrier and engage themselves in morphogenetic signal transduction pathways at the epithelial surface.\textsuperscript{[16,17]} Mucin glycosylation content dictates the biochemical and biophysical properties of visco-elastic secretions, pointing out an important

Table 1: Distribution of immunohistochemical score among the study groups

| Group                              | Mean | SD  | SEM | 95% CI for mean | Minimum | Maximum | P       |
|------------------------------------|------|-----|-----|-----------------|---------|---------|---------|
| Oral squamous cell carcinoma       | 44.00| 9.28| 2.08| 39.65–48.35     | 25      | 58      | <0.001* |
| Potential malignant disorder       | 28.00| 15.62| 3.49| 20.69–35.31     | 0       | 48      |         |
| Normal oral mucosa                 | 0.75 | 2.45| 0.55| −0.40–1.90      | 0       | 10      |         |

*Significant difference. SD: Standard deviation, SEM: Standard error of mean, CI: Confidence interval

Figure 2: Photomicrograph of normal oral mucosa for MUC1 mucin (IHC stain, x100)

Figure 3: Photomicrograph of mild epithelial dysplasia for MUC1 shows cytoplasmic staining from basal to spinous layer of epithelium (IHC stain, x100)

Figure 4: Photomicrograph of severe epithelial dysplasia shows faint positivity for MUC1 epithelial cells (IHC stain, x100)

Figure 5: Photomicrograph of oral submucous fibrosis showing positivity for MUC1 in epithelial cells (IHC stain, x40)
In malignant neoplasms, aberrant mucin expression and changes in phosphorylation correlates with the difference in cell adhesion.\cite{7,8,10,17} In malignant neoplasms, aberrant glycosylation of MUC1 often leads to a reduction in the length of the carbohydrate chains and exposes normally cryptic antigens of peptide and carbohydrate nature that make MUC1 epitopes tumor-specific.\cite{5,8,10} MUC1 mucin expression may be related to the invasion or metastasis of carcinoma cells.\cite{12} The membrane and cytoplasm staining of MUC1 in the squamous cells might correspond to its transmembrane and cytoplasmic subunits, respectively.\cite{10,13} A study conducted by Nitta et al.\cite{12} using MUC1 and Narashiman et al.\cite{6} with MUC4, showed positivity in the OSCC samples which was highly restricted to the well-differentiated areas and the keratin pearls of the tumors. A similar correlation was seen in our study.

Overexpression of MUC1 in OSCC cells compared with its normal and PMD’s counterpart clearly suggests role of MUC1 in the pathogenesis of OSCC, as seen in a study conducted by Nitta et al.\cite{12} and Narashiman et al.\cite{6} Further the cellular expression of MUC1 showed a steady increase from dysplastic noninvasive lesions to invasive OSCC.\cite{12} Localization and identification at the ultra-structural level of MUC1 mucin in OSCC may provide important information on the role of glycoproteins in cellular malignant transformation.\cite{11,12}

In this study, the age presentation of OSCC ranged from 27 to 76 years, with the mean age of 45.8 years. Gender distribution was 9 (45%) men and 11 (55%) women. Out of twenty OSCC cases 7 (35%) showed poorly differentiated OSCC and 13 (65%) showed well-differentiated OSCC. The age presentation of PMD’s ranged from 26 to 70 years, with the mean age of 43.9 years. Gender distribution was 13 (65%) men and 7 (35%) women. Among leukoplakia cases, 2 (20%) showed mild epithelial dysplasia, 3 (30%) moderate epithelial dysplasia and 5 (50%) severe epithelial dysplasia [Table 2].

Initial studies showed that MUC1 was phosphorylated on both tyrosine and serine residues within the cytoplasmic tail and changes in phosphorylation correlates with the difference in cell adhesion.\cite{7,8,10,17} In malignant neoplasms, aberrant glycosylation of MUC1 often leads to a reduction in the length of the carbohydrate chains and exposes normally cryptic antigens of peptide and carbohydrate nature that make MUC1 epitopes tumor-specific.\cite{5,8,10} MUC1 mucin expression may be related to the invasion or metastasis of carcinoma cells.\cite{12} The membrane and cytoplasm staining of MUC1 in the squamous cells might correspond to its transmembrane and cytoplasmic subunits, respectively.\cite{10,13} A study conducted by Nitta et al.\cite{12} using MUC1 and Narashiman et al.\cite{6} with MUC4, showed positivity in the OSCC samples which was highly restricted to the well-differentiated areas and the keratin pearls of the tumors. A similar correlation was seen in our study.

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In this study, statistically significant difference in mean immunohistochemical score was observed between OSCC and PMD's group ($P < 0.01$), OSCC and NOM group ($P < 0.001$) as well as between PMD’s group and NOM group ($P < 0.001$). [Table 1 and Graph 1].

Nitta et al. in their study showed statistically significant difference between NOM and epithelial dysplasia ($P < 0.01$), between NOM and carcinoma in situ ($P < 0.01$), between NOM and OSCC ($P < 0.01$), and between epithelial dysplasia and OSCC ($P < 0.01$). Dominant cytoplasmic expression was found be increasing from premalignant to malignant lesions ($P < 0.001$).[12]

CONCLUSION

The present study infers up-regulation of MUC1 mucin expression in PMD’s and malignant lesions might play a vital role in the pathogenesis and its progression. It can also be a useful diagnostic marker for prediction of the invasive/metastatic potential of OSCC. Hence, MUC1 mucin can be regarded as a useful marker for PMD’s and OSCC.

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Conflicts of interest
There are no conflicts of interest.

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