Original Article

MUC4 Expression in Oral Dysplastic Epithelium and Oral Squamous Cell Carcinoma: An Immunohistochemical Study

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ABSTRACT

Objective: MUCIN4 (MUC4) glycosylation is linked to the oncogenesis and progression of a neoplastic process. It can suggest information pertaining to tumor progression, management and its natural properties. Thus, MUC4 can play a pivotal role in prognostic diagnosis. This study aimed to analyze the MUC4 expression in oral cell squamous carcinoma and oral dysplastic epithelium. **Materials and Methods:** The research included 45 samples of oral epithelial dysplasia (OED) and 45 cases of oral squamous cell carcinoma (OSCC). In order to carry out the investigation, tissue blocks of previously diagnosed cases of OED and OSCC were retrieved from the relevant archives. Forty-five OED cases were categorized into three group's mild, moderate and severe dysplasia, with 15 cases in each respective category. Forty-five OSCC cases were categorized into three groups: well differentiated, moderately differentiated, and poorly differentiated OSCC with 15 cases in each respective category. Ten tissue biopsies of normal oral mucosa were obtained from subjects in the control group. The chi-square test and one-way ANOVA were used for statistical analysis. **Result:** There was an absence of MUC4 expression in normal mucosa, whereas the OED and OSCC groups had a significant amount of observable variance. Within the OED category of cases, a consistent progression from mild to severe dysplasia was seen in terms of the staining pattern. Cases with severe dysplasia displayed a staining pattern that covered the complete thickness of the tissue in the epithelium. Expression of MUC4 was shown to be lower in moderate differentiated squamous cell carcinoma (MDSCC), and poorly differentiated squamous cell carcinoma (PDSCC) as compared to well differentiated squamous cell carcinoma (WDSCC). It showed decreasing pattern across all grades of OSCC. In WDSCC, an intense highest staining response was noticed, particularly among the cells that are highly differentiated and take the form of a honeycomb pattern. **Conclusion:** Analysis of the expression profile of MUC4 and the aberrant expression of this gene in OSCC suggests that it may serve as a useful diagnostic marker. Therefore, it is possible to draw the conclusion that MUC4 plays a very significant part in the pathogenesis of OSCC and also acts as a marker that may be taken into consideration for the accurate diagnosis of OED and OSCC.

Keywords: *Muc4, oral leukoplakia, oral squamous cell carcinoma*

Introduction

The term "Epithelial dysplasia" denotes the microscopic abnormalities present in OPMD/

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OSCC. It indicates a higher risk of malignant transformation, after which a grade of severity is assigned.[1-3] There is a strong connection between more severe grades of dysplasia and an elevated likelihood of developing cancer.[4-6] These kinds of lesions are considered to be potentially malignant conditions because of the intrinsic disparity that exists between them.[7,8] Dysplasia is subdivided into a number of grades according to architectural and cytological criteria, the majority of which are used by oral pathologists to arrive at their diagnoses after taking into account the interexaminer and intraexaminer variability that can exist in their findings.[9,10] Various markers have been investigated, and among them, mucin has the potential to be put forth as a viable biomarker or indicator.[11,12] Mucins are glycoproteins that are either membrane-bound or membrane-secreted, and their structure is determined by the expression of a gene located on chromosome 3q29 in epithelial cells.[13] A variety of human neoplasms have been shown to have invariantly high levels of MUC4 expression, and the interaction between these two proteins generates a positive intonation of the semaphore, which results in uncontrolled growth and constitutive survival.[14] Thus, MUC4 has the potential to become an essential diagnostic tool for the diagnosis, progression, and therapy of malignancies.[15] The present investigation is an idiosyncratic study that is performed to analyze the expression of MUC4 in various grades of oral dysplastic epithelium and oral squamous cell carcinoma (OSCC) along with normal tissue.[16] A handful of Studies that were carried out revealed the MUC4 aberrant expressions in squamous cell carcinoma that affects the oral cavity. This study aimed to analyze MUC4 expression in various grades of oral dysplastic epithelium in OSCC and normal tissue.[16]

Materials and Methods

This study is a retrospective analysis aimed at evaluating and comparing MUC-4 expression in OED and OSCC. Ethical committee clearance was obtained from institutional ethical clearance committee Sri Sai College of dental surgery oral pathology department, Vikarabad, Hyderabad (Ethical number Ref.No.12/PHD/SSCDS/IRB-E/2018).

Estimates of the sample size were calculated with the help of the G power software. Forty-five OED cases were categorized in three group's mild, moderate and severe dysplasia with 15 cases in each respective category. Forty-five OSCC cases were categorized into three groups: well differentiated, moderately differentiated, and poorly differentiated OSCC with 15 cases in each respective categories. Control samples consisting of 10 samples of healthy oral mucosa were also included.

The procedure was carried out using biopsied tissue samples that had already been analyzed and shown to contain OSCC and OED. These samples were taken from the collections of the Pacific Dental College at Pacific University in Rajasthan, the Mehdi Nawaj Jung Institute of Oncology in Hyderabad, and Sri Sai College of Dental Surgery. Paraffin embedded tissue slices were examined.

The inclusion criteria of the study were patients who have been diagnosed with OED both clinically and histopathologically Patients who have been diagnosed with OSCC both clinically and histopathologically Control groups consisting of normal healthy people. The exclusion criteria of the study were patients who have developed oral cavity metastases from a secondary squamous cell carcinoma and the patient receiving both chemotherapy and radiation treatment.

The healthy patients comprised of patients undergoing third molar surgery and extractions for correction of teeth provided the normal oral mucosa specimens that were used for the study's control group. After cutting successive slices from the blocks with diameters ranging from 3 to 4 microns, the sections were stained with the assistance of an immunohistochemical marker for MUC4.

Immunohistochemistry procedure

Immune histochemical techniques were procured from Pathnsitu Biotechnologies Pvt. Ltd equipped to wield kit which encompasses:

The primary antibody is an antihuman mouse IgG. Metallothionein Antimouse IgG serves as the secondary antibody. Peroxidase Block Conjugate – Horse Radish Peroxidase Chromogen substrate – Diaminobenzidine tetra hydrochloride (DAB)

Staining procedure

Formaldehyde Slides coated with 3-aminopropyl triethoxy silane (APES) were used to hold fixed paraffin-embedded tissue slices of 3–4 m thickness that had been heated for 1 h at 60°C. Three alters of Xylene treatments for 5 minutes were used for deparaffinization, after which, for rehydrating, we used progressively weaker grades of alcohol concentrations (100% alcohol for 3 min, 90% for 3 min, and 80% for 3 min). Endogenous peroxide block was done using 3% hydrogen peroxide, followed by washing in distilled water and TRIS buffer to prevent further endogenous peroxide blocking. Preheating at EDTA buffer (ph-8.0) at 450W for ten minutes, three cycles of Microwave treatment of slides

in EDTA buffer at 600W for 10 minutes, were done to retrieve antigens. Subsequently, TRIS buffer was used to wash the slides. [Ph-7.6]. Sections were then incubated with primary anti-MUC4 monoclonal antibody for 1 hour and 30 minutes and then washed in TRIS buffer. After being washed alongside TRIS buffer, tissue slices ensue incubated with polyexcel polyHRP from the secondary antibody kit at room temperature for 10 minutes. A DAB chromogen incubation period of 5 minutes was performed on the slides. Next, Tris buffer was used to clean the slides. Hematoxylin was used as a counterstain, and the slices were then washed under running water. After the slides dried, they were cleaned, and DPX was used to mount them.

Sectioning

To ensure good adherence & observation of the section to the slide, sections measuring 3-4 microns in thickness were obtained and placed on poly-L-lysine adhesive-coated slides. The slides were then placed in a slide warmer and heated to temperatures between 50 and 60 degrees centigrade for three hours.

Evaluation of staining

MUC4 positive cells were evaluated at 10x and 40x magnification using a double-headed light microscope. In mitotic nuclei, brown staining identified MUC4-positive cells. Ten randomly selected fields had 100 cells counted from each.

Evaluation

The MUC4 marker LIs for NOM, OED, and OSCC were calculated using the provided formula. Semi-quantitative analysis of MUC4 expression was performed using the labelling index (LI) = labelled cells/total cells x 100. (percentage of cells that were positive). A zero indicates negative results, that is, no immune-stained cells present. A score of +1 if there are 25% or fewer immune cells, a score of +2 (between 25% and 50%), and +3 indicates a "strong" result (i.e., >50%).

Statistical analysis

All of the computations were carried out with the assistance of the statistical software program known as IBM SPSS 20.0. In order to be promulgated that it has statistical significance, the p-value must be lower than 0.05. The variability between observers was analyzed using the Pearson correlation, and the correlation within the group was determined with an analysis of variance followed by a post hoc Tuckey test. The data derived from the study were subjected to the Chi-square test and one-way ANOVA.

Results

The MUC4 staining was evaluated by two independent observers. The interobserver reliability in recording the Muc4 expression was 0.634, which was statistically significant at the 0.000 level, indicating that the two observers were in agreement. Hence, the observations made by observer 1 were taken into account for the subsequent statistical analysis. The MUC4 expressions in both the control group and the research group were evaluated using the IRS scoring system. According to the expression pattern, IRS assigns a low or a high score to the observation. Low expression was defined as an IRS score of less than 3, while strong expression was defined as an IRS score of more than 3. Only one of ten normal mucosa tissue samples of the control group showed immunostaining for MUC4, and the percentage of positive cells that were seen was less than 20%. All other biopsies showed a lack of positive staining [Figure 1]. In mild dysplasia, in nine cases (out of 15 total cases) the percentage of positive cells was less than 10%, whereas in three cases it was between 10% and 50%. While six of the moderate dysplasia (out of 15 total cases) exhibited less than 10% of positive cells, the other nine cases showed between 16% and 42% of immune positive cells. On the other hand, in severe instances of dysplasia, the proportion of positive cells was between 10% and 50% in 10 cases, while less than 10% in other cases [Figures 2–4]. Out of 15 cases of WDCC carcinoma examined, 10 exhibited 10%–50% positive cells, 2 cases showed 50%–80% positive cells, and 2 cases showed less than 10% positive cells [Figure 5]. Four of the seven cases with MDSCC had a proportion of positive cells between 10% and 50%, two cases had between 50% and 80%, and seven cases had less than 10% [Figure 6]. In PDSCC, however, only two of the 15 cases exhibited 50% positive cells, while one case showed fewer than 10% positive cells [Figures 6–8]. Six of the 15 cases of mild dysplasia displayed mild staining intensity, and five cases showed a moderate response, according to the staining intensity score that followed the IRS grading. The responses was mild in 7 cases of moderate dysplasia, and 7 cases of severe dysplasia and moderate in 6 cases of moderate dysplasia and 7 cases of severe dysplasia. Measurements of staining intensity revealed that WDSCC cases exhibited a more intense staining pattern, with 10 cases exhibiting a moderate response and one case displaying a mild reaction. However, in MDSCC and PDSCC, the intensity dropped, with 7 cases and 1 case showing a moderate response, 5 cases and 2 cases showing a mild reaction, and 12 cases of PDSCC showing no change in color at all. Ten cases

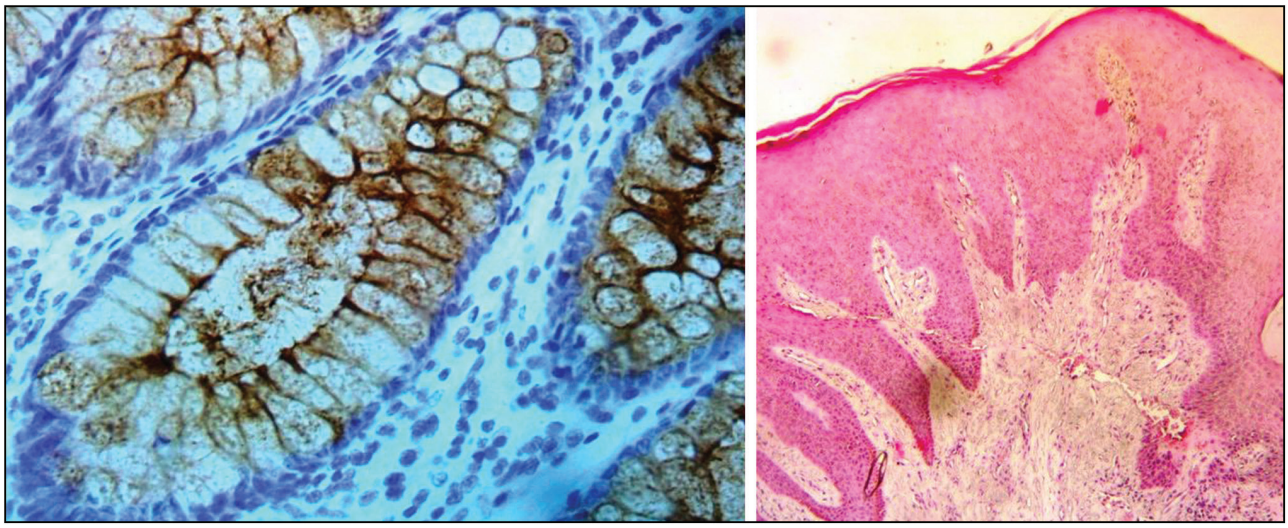

Figure 1: Photomicrograph colon tissue membrane staining (Muc4 40×) and oral mucosa hematoxylin and eosin section (10×) positive control

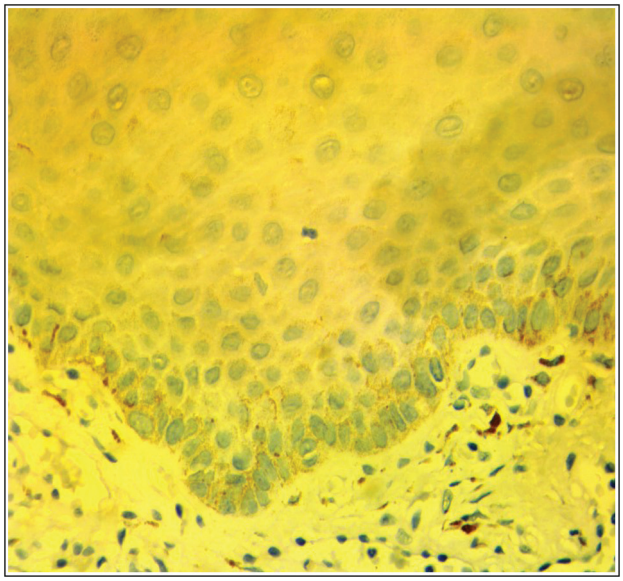

Figure 2: Photomicrograph Muc4 immunostaining in basal layers of mild dysplasia (40×)

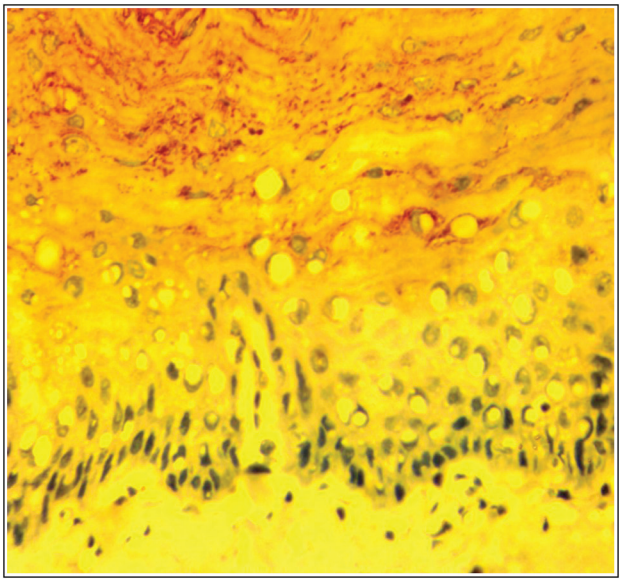

Figure 3: Photomicrograph 4Muc4 immunostaining in suprabasal layers and moderate dysplasia (40×)

of mild dysplasia were deemed as negative, two cases as mild, and three cases were deemed moderate using the IRS 4-point assessment from the OED. Five cases with moderate dysplasia were classified as mild, 4 cases as moderate, and 6 cases as negative. Five of the severe dysplasia cases were deemed negative, three were classified as mild, and seven cases were classified as moderate [Table 1]. On the contrary, in OSCC, 11 cases of WDSCC were evaluated as moderate, 3 cases were classed as mild, and 1 case was graded as negative. Although there was a reduction in expression instances of MDSCC and PDSCC, only four cases and one case, respectively, met the criteria for moderate severity. Pearson's chi-square test was used for the intragroup analysis that was performed within the OED group. Because of this, it was possible to determine the IRS score for dysplasia ranging from mild to severe. According to the results of the chi-square test, 33.3% of cases with mild, moderate, and severe dysplasia had a low IRS score, while 33.3% exhibited a high IRS score. Additionally, 33.3% of cases had a moderate IRS score. The *P*-value that was obtained was 1.00, which indicated that there was no statistical significance. However, the results of Pearson's chi-square test were used in order to determine the OSCC IRS score for WDSCC, MDSCC, and PDSCC [Table 2]. Based on

the findings obtained through the chi-square test, it was determined that 16.1% of the WDSCC had a low IRS score, while 71.4% had a high score. With respect to MDSCC, the value obtained for low and high scores was 38.7% and 21.4%. In PDSCC, Low IRS values of 45.2%, and high IRS values were only 7.1%. The p-value that was obtained was 0.001, which indicated it was statistically significant. Only 4.3% of instances in the control group exhibited low MUC4 expression, while the remaining cases did not demonstrate any change in color when the MUC4 immunostaining was analyzed in both the control and study groups when comparisons of the two groups were made. In the study sample, low expression comprises 22.6% of OED cases, while high expression comprises 17.6% of OED cases. The percentage of OSCCs with high MUC4 expression got elevated to 82.4%, whereas the percentage with low MUC4 expression fell to 58.5%. Pearson's chi-square test was used to compare MUC4 immunostaining intensity between the control and study groups. Results showed statistical significance at a *P*-value of 0.003 [Table 3].

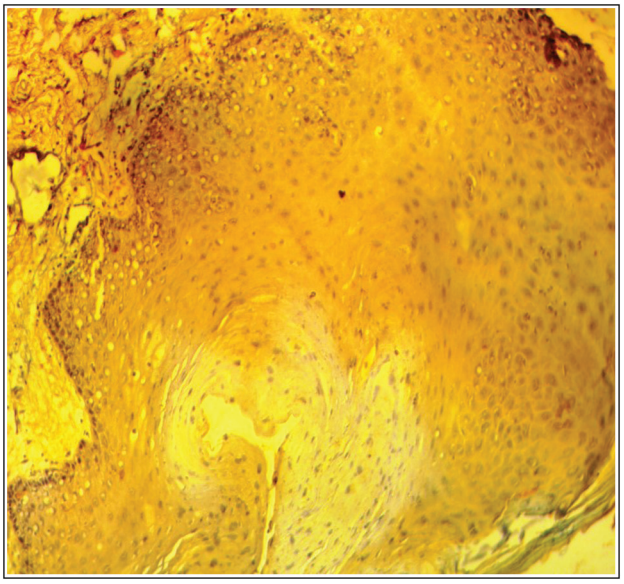

Figure 4: Photomicrograph 5Muc4 immunostaining in full thickness of the epithelium and severe dysplasia

Figure 6: Photomicrograph Muc4 immunostaining in MDSCC

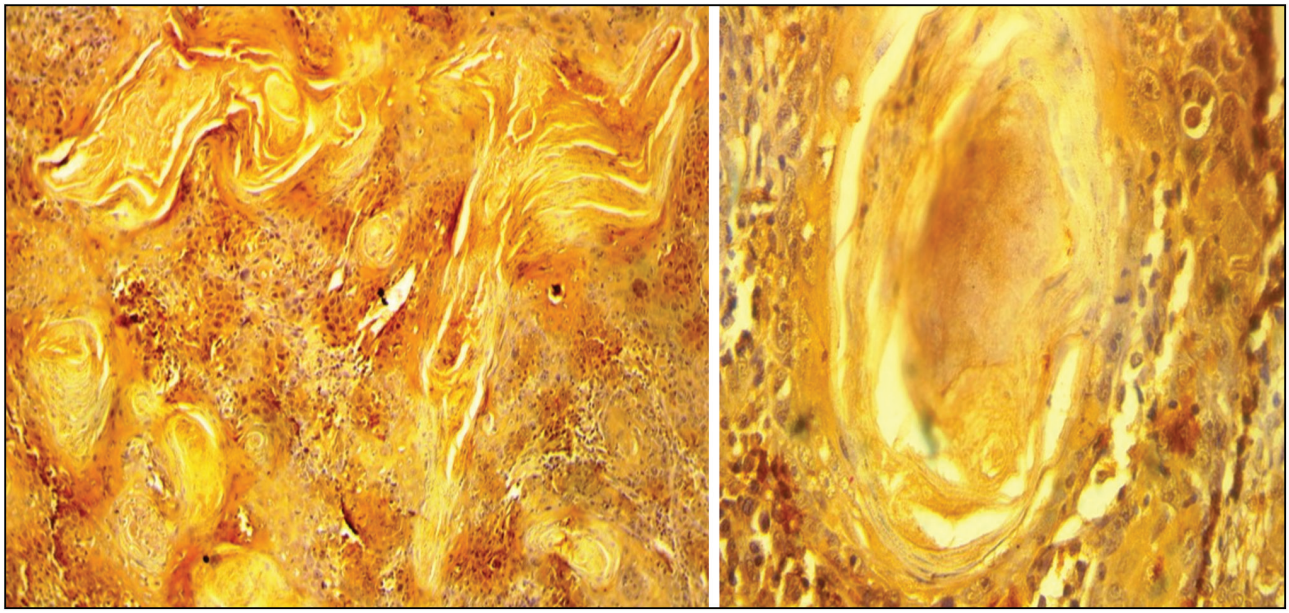

Figure 5: Photomicrograph displaying MUC4 immunostaining in WDSCC (10×) and keratin pearl (40×)

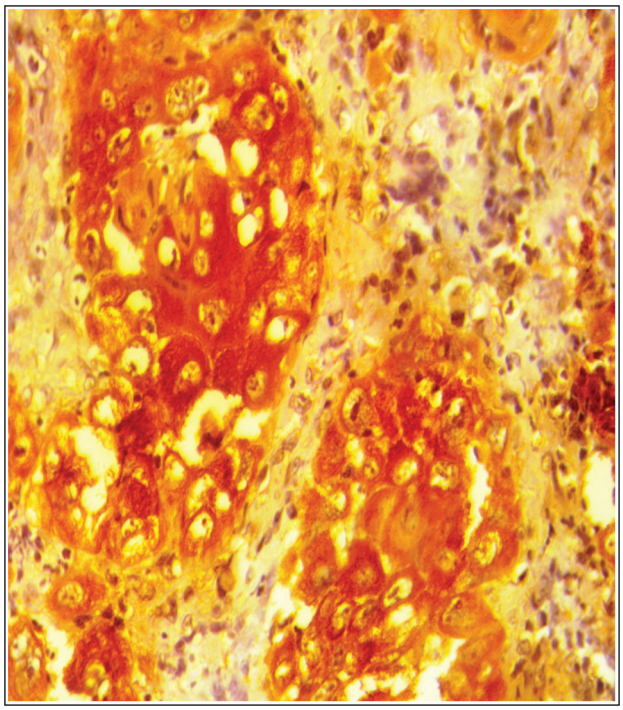

Figure 7: Photomicrograph Muc4 immunostaining in well-differentiated cells in MDSCC (40×)

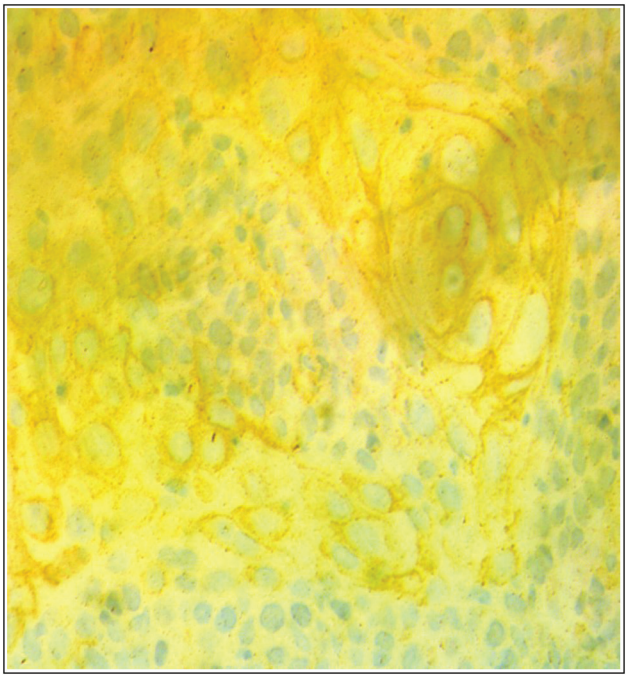

Figure 8: Photomicrograph Muc4 immunostaining in PDSCC (40×)

Table 1: IRS score of MUC4 in OED

Group	*N*	Final IRS score			
		0–1 = negative	2–3 = mild	4–8 = moderate	9–12 = strongly +ve
Mild dysplasia	15	10	2	3	0
Moderate dysplasia	15	6	5	4	0
Severe dysplasia	15	5	3	7	0

Table 2: IRS score of MUC4 IN OSCC

Group	*N*	Final IRS score			
		0–1 = negative	2–3 = mild	4–8 = moderate	9–12 = strongly +ve
WDSCC	15	1	3	11	0
MDSCC	15	2	9	4	0
PDSCC	15	11	3	1	0

Table 3: MUC4 immunostaining in control and study group

Categorization	N	MUC4 expression[a]		*P* Value[b]
		Low	High	
Control	10	4.3%	0.0%	0.003
OED	15	22.6%	17.6%	
OSCC	45	58.5%	82.4%	

Chi-square test

[a]MUC4 expression, high means IRS score > 3 and low means IRS score < 3

[b]$P < 0.005$, statistically significant

Discussion

This study found elevated MUC4 expression in dysplasia and OSCC but no expression at all in normal mucosa. Evaluation using MUC4 was performed in recent times for a variety of cancers, including adenocarcinoma of the esophagus, Crohn's disease, and carcinomas of the gall bladder.[12-19] In a case study of leukoplakia and OSCC carried out by Narasimhan *et al.*,[10] the researchers found that MUC4 expression was not present in the normal oral mucosa. Hamada *et al.*[11] used immunohistochemistry to study the MUC4 expression lineament in OSCC tissues. They found that MUC4 staining was not present in all normal squamous epithelium. This finding was based on their observation that OSCC tissues had a distinct pattern of MUC4 expression. In patients with normal oral mucosal epithelium, MUC4 positive was only seen in 4% of the total patients. The study carried out by Xiao Peng Gao (2021)[12]regarding MUC4 expression pattern in Pan Cancer investigated that the expression sequence and prognostic value OF MUC4 in pan cancer. The study showed that MUC4 expression and methylation status are very strong prognosis indictors for carcinoma of lungs along with its diverse properties could be utilize as a handy tool in studying the carcinoma affecting the pancreas. The study observation throws a bright light on the biological understanding of MUC 4 &

developing therapeutic strategies. Only 10% of normal tissues were positive for Muc4 expression, according to a study by Macha *et al.*[13] that examined the functional role of MUC4 in HNSCC developing cell lines. The fact that MUC genes have relatively tissue specific expression helps to explain our finding along with the above mentioned study's conclusion, as well as the findings of the aforementioned investigations. An increase in the immunostaining of MUC4 was found to be consistent across all grades of OED that were evaluated in our study. In situations of mild dysplasia, the expression was only seen in the lower basal cell layers and above the supra basal layers, however in cases of severe dysplasia, the staining extended all the way up to the granular layer of the epithelium. A pattern of expression that was similar to that seen with severe dysplasia was identified with all of the cases displaying staining over the whole epithelial thickness. As a result, mucin is put to use as an important biological marker in order to differentiate between healthy and diseased states of being. In the current study, in comparison to the OED group, a distinct difference in the expression of MUC4 was observed, which is in agreement with the research that was cited earlier. The results of this study are comparable to those found in the studies carried out by Narasimhan *et al.*,[10] Hamada *et al.*,[11] Kong,[15] and Munro *et al*.,[16] respectively. Narasimhan *et al.* [16] conducted research on the expression of MUC4 in leukoplakia cases and found that the staining pattern gradually became more severe as the disease progressed from mild to severe dysplasia. In a different study that was carried out on OSCC tissues by Hamada *et al.*,[11] abnormal MUC4 expression was observed in cancer adjacent dysplastic epithelium. The research that was carried out by Kong[15] looked at the differences in mucin expression that were associated with the various squamous dysplastic transformations of exocervical epithelium that can occur in benign and malignant cervical lesions. According to the findings of their research, MUC4 and MUC20 were found to be present in cervical dysplasia. Cervical dysplasia contained a greater number of MUC4-expressing cells than squamous metaplasia did, and the levels of MUC4 and MUC20 found in squamous metaplasia cells were lower. When compared to normal exocervical and endocervical epithelia, moderate and severe dysplasia had the highest number of positive cells detected, whereas normal exocervical and endocervical epithelia displayed minimal MUC4 expression. There was a direct correlation found between the positive rates of MUC4 and MUC 20 and the degree of differentiation as well as the clinical stage of squamous cell carcinoma. The fact that Muc4 is expressed in OSCC in a manner that is different from other cancers is one of the findings that stand out most from this research. The findings of the current study revealed abnormal levels of MUC4 expression in varying degrees of OSCC. According to the results of the evaluation of the IRS scores for the WDSCC group, the majority of the cases displayed moderate expression, while the remaining cases displayed mild expression. MUC4 positivity was seen as the predominant staining pattern in WDSCC samples. This positivity was primarily confined to regions of the tumor that were already well differentiated and contained squamous keratin pearls. Few cases displayed entire thickness staining of the epithelium; however, in almost all the cases, intense staining of the epithelium was seen in a honeycomb pattern the other MDSCC instances had a milder kind of manifestation. In the MDSCC histological grade, only four cases displayed moderate expression of MUC4 of fifteen cases. The rest of the MDSCC cases displayed mild expression. As the histological grade of OSCC continued to rise, it was found that only four out of fifteen instances of PDSCC had a weak expression, while the other cases all displayed a negative expression. Expression of MUC4 was shown to be lower in WDSCC, MDSCC, and PDSCC as compared with WDSCC. This was the case across all grades of OSCC. Only differentiated cells produced mucins, as shown by the expression pattern in MDSCC and PDSCC, and this was shown by the staining response in PDSCC, which showed very little staining.[17] Staining pattern of WDSCC group in this study correlates with MUC4 expression studied in non-small lung cell carcinomas (NSCLC) by Kwon.[17] Although Workman *et al*.[18] observed lower expression levels of MUC4 in initial tumors in comparison to normal breast tissue, they noted that higher levels of MUC4 were present in instances of breast cancers that had spread to lymph nodes via lymph node metastasis. Because of this, they came to the conclusion that the expression of MUC4 is a hallmark for completely differentiated breast epithelium, and that dedifferentiated breast tumor cells are unable to maintain the production of MUC4 properly. This finding holds true for both our research and the previous one, in which the WDSCC group demonstrated the greatest amount of staining response restricted to keratin pearls. Our results are consistent with those of research that was carried out by Tamura *et al.*[19] utilizing MUC4 antibody in both non-neoplastic gastric mucosa and gastric cancer. They made the observation that in non-neoplastic gastric mucosa, MUC4 was very seldom seen in the cytoplasm of the surface mucous epithelium. However, they found that MUC4 was regularly present in the cytoplasm of fundic

and pyloric glands, although with a variable degree of certainty. Upon closer inspection, the instances of gastric adenocarcinoma revealed varying degrees of expression, depending on their particular histological subtype. In the case of the highly differentiated adenocarcinoma, the pattern of staining was quite noticeable and conspicuous. This finding was made in relation to the fact that OSCC has a different pattern of expression than other cancers. The researchers concluded that the WDSCC cases were more likely to show intense staining pattern. The findings of the present investigation are comparable to those of the study that was discussed before. Their findings led them to the conclusion that a decrease in MUC4 expression in MDSCC could be attributed to the inability of less differentiated squamous cells to express MUC4, in contrast to the ability of well differentiated OSCC cells to express MUC4. This conclusion was reached on the basis of their findings. In the research carried out by Hamada *et al.*,[11] an assessment of the muc4 expression profile revealed that it was present in the carcinoma cells of 41% of OSCC patients. This was the case despite the fact that the majority of normal squamous epithelium lacked the expression. Macha *et al.*[13] examined the expression of MUC4 in human HNSCC tissues. Their findings indicate that MUC4 is not controlled in HNSCC tumors, which brings up the idea that an excessive amount of Muc4 may contribute to the development of HNSCC. In their study of immunohistochemical coexpression of muc1 and muc4 in oral leukoplakia and OSCC, Rathee *et al.*[20] established MUC4 as prognostic markers. They found that the expression of these markers increased from normal mucosa to oral leukoplakia and then to OSCC, which is in line with the findings of our study. Kumar *et al.*[21] in their institutional study observed that there is mean MUC1 positive cells 40%bin OSCC, 28% IN PMD & 0.75% IN NOM. Increased IHC score was seen in OSCC group followed by PMD group & NOM group. The difference in Immunohistochemical score among the groups was statistically significant. In their work, Thakur *et al.*[22] observed an elevation of MUC1 in OSCC patients. This finding lends credence to the hypothesis that MUC1 likely plays an important role in the pathogenesis and development of OSCC, in addition to its role as an early diagnostic marker. Lu *et al.*[23] conducted a comprehensive meta-analysis study in which they evaluated the predictive relevance of mucin expression in head and neck cancer in a systematic manner (HNC). According to the findings of the meta-analysis, mucin expressions were linked to a less favorable overall survival rate, a headway TNM stage, towering metastasis of lymph nodes, and penetration in the underlying tissue to a far extent.

MUC1 expression was shown to be present in ESCC tissues by Sun *et al.*,[24] who also discovered that the presence of MUC1 was significantly noteworthy in malignant tissue compared to paracancerous normal tissue. This study's findings primarily showed that MUC1 expression might be used as a sole predictive factor, and it also showed a correlation with the metastatic capability of the tumor, which was associated with a high risk of recurrence postoperatively in patients with ESCC.

Consistent with previous research, our study found elevated Muc4 expression in dysplasia and OSCC but no expression at all in normal mucosa. Unfortunately, the function of muc4 in oral squamous cell cancer has been the subject of very few investigations. Overexpression of Muc4 may have a role in the etiology of HNSCC, as shown by our findings. Data from the current investigation, as well as the aforementioned literature, point to a crucial part played by MUC4 in the nascent phases of carcinogenesis when squamous dysplastic epithelium is transformed into OSCC. However, in order to identify Muc4's role in the development of tumors, further research is required.

Conclusion

The current research work has assisted us in arriving at an extremely significant conclusion, which is that MUC4 plays an essential part in the pathoses of OSCC and has the potential to serve as a diagnostic tool or biomarker for the early detection of OED and OSCC. However, in the future, research with a larger number of participants and a broader scope need be carried out in order to demonstrate that MUC4 is a highly effective pragmatic diagnostic and prognostic tool.

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Conflicts of interest

Authors declared no conflicts of interest.

Authors contributions

The authors confirm contribution to the paper as follows: study conception and design, Mohammed Abidullah; data collection, Prashanth Nahar; analysis and interpretation of results, Syed Afroze Ahmed; draft manuscript preparation, Hemant Kothari and Sana Vakeel. All authors reviewed the results and approved the final version of the manuscript.

Ethical policy and Institutional Review board statement

Ethical committee clearance was obtained from institutional ethical clearance committee of Sri Sai College of Dental Surgery, Oral Pathology Department, Vikarabad, Hyderabad (Ethical Number Ref. No. 12/PHD/SSCDS/IRB-E/2018).

Patient declaration of consent

Study was explained to the patient and patient consent was taken.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon request.

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