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HISTOCHEMICAL ANALYSIS OF STROMAL MUCIN: A COMPARATIVE STUDY ACROSS GRADED STAGES OF ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Background: Mucins are key stromal glycoproteins whose alterations play a role in oral carcinogenesis. Evaluating their distribution may provide insights into the progression from Oral Epithelial Dysplasia (OED) to Oral Squamous Cell Carcinoma (OSCC).

Aim: To compare stromal mucin expression in various grades of OED and OSCC using Haematoxylin & Eosin (H&E) and Periodic Acid-Schiff (PAS) stains.

Materials and Methods: Fifty histopathologically confirmed cases (25 OED, 25 OSCC) and 10 salivary acini controls were studied. Sections (4 µm) were stained with H&E and PAS. Mucin distribution, staining pattern, and intensity were analysed in 10 fields per case.

Results: Results revealed a progressive decline in acid mucin expression from mild to severe OED, with weak and focal positivity in OSCC. Conversely, neutral mucins demonstrated a gradual increase in intensity from OED to OSCC, particularly around tumour islands, reflecting extracellular matrix (ECM) remodelling and tumour progression. PAS staining showed higher intensities in dysplasia compared to OSCC, while H&E staining highlighted greater cytological atypia in severe dysplasia and well-differentiated OSCC.

Conclusion: These findings suggest dynamic shifts in mucin profiles during oral carcinogenesis, underscoring their potential as histopathological markers for distinguishing pre-invasive and invasive lesions. The study highlights the diagnostic value of mucin histochemistry in understanding tumour progression and provides a cost-effective adjunct to advanced molecular techniques.]

INTRODUCTION

Mucins are mucopolysaccharides, major glycoprotein component of stromal matrix. Mucins are heavily O-glycosylated linear glycoprotein synthesized and secreted predominantly by epithelial cells lining various mucosal surfaces and glandular structures throughout the body. Alterations in mucin production and expression have been implicated in several diseases, including cystic fibrosis and certain malignancies such as those affecting the oral cavity, gastrointestinal tract, and breast[1]. Tumour cells can often produce altered forms of mucins, which can help them evade immune detection or facilitate metastasis. In most of

carcinoma types tissue overexpress mucins to exploit their role in survival and growth. This overexpression and modification of mucins are hypothesized to contribute critically to tumour cell survival, proliferation, and invasion, thus representing potential biomarkers and therapeutic targets in carcinoma biology.

Several studies have been done to study the neoplastic changes in tumor progression. Main focus of this study will be to evaluate the alterations of mucins in various grades of Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC) and will observe the staining intensity and pattern of glycoproteins i.e. acid mucin, neutral mucin. Utilizing a panel of histochemical staining methods, this study aims to delineate the differential distribution and intensity of acid and neutral mucins, thereby offering insights into mucin remodelling during oral carcinogenesis. This study will also compare efficacy of two stains in study of various grades of OED and OSCC.

Oral Squamous Cell Carcinoma (OSCC) initiates its development from oral epithelial dysplasia (OED), which is characterized by abnormal cell growth in the oral mucosa[2]. As the dysplastic process advances, significant alterations occur within the extracellular matrix (ECM) and the basement membrane—the thin, fibrous layer that separates the epithelium from the underlying connective tissue. These changes facilitate the transition from a benign condition to a malignant one, as dysplastic cells not only proliferate but also invade surrounding healthy tissues.[3] This invasion triggers a cascade of reactive alterations within the stroma, which includes inflammatory responses and modifications in the ECM composition. Such changes can further promote tumour progression by allowing for greater tumour cell migration and metastasis, ultimately contributing to the aggressiveness and poor prognosis commonly associated with OSCC.[4]

The phenotypic changes observed in carcinoma are influenced not only by genetic mutations within tumour cells but also by the surrounding tumour microenvironment (TME). The tumor microenvironment (TME) encompasses various components, including blood vessels, fibroblasts, immune responses, signaling pathways, signalling molecules and the extracellular matrix (ECM).[5] The process of carcinogenesis and its subsequent progression are the result of complex interactions between these two elements, highlighting the significance of both the tumor cells and their microenvironment in the development of cancer. Carcinogenesis and its progress are a defective result of both parts.[6]

Materials and Methods:

In this study, a total of 50 histopathologically confirmed cases of Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC) were retrieved from paraffin-embedded specimen archives. Of these, 25 cases were OED and 25 were OSCC. Within the OED group, 8 cases showed mild dysplasia, 9 showed moderate dysplasia, and 8 showed severe dysplasia. Among the OSCC cases, 8 were well-differentiated, 7 were moderately differentiated, and 10 were poorly differentiated. Additionally, 10 control samples of salivary acini were included.

From each specimen, 2 sections of 4 µm thickness were prepared. One section was stained with Haematoxylin & Eosin, and the other with Periodic Acid-Schiff (PAS) stain, following standard protocols. OED and OSCC cases were graded by a single observer according to the WHO (1978) classification and Broder's system (1920).

Following staining and fixation, the stromal mucin architecture was examined by analysing 10 randomly selected fields under 10x and 40x magnification. Mucin distribution patterns were categorized as either focal or diffuse. The predominant colour of the mucins was noted, and staining intensity was scored as follows: 0 = absent, 1 = minimal, 2 = weak, and 3 = bright.

STAINING INTENSITY	SCORE
Absent	0
Minimal	1
Weak	2
Bright	3

Statistical analysis

IBM SPSS Corp. in Armonk, New York for Windows, Version 25.0, was used for the statistical analysis. Descriptive statistics were computed and presented in terms of Mean and Standard Deviation. One-way ANOVA statistics were applied to calculate the inferential statistics between the different groups. Post Hoc Turkey HSD statistics were used to compare multiple groups. The statistical constant was fixed at $p<0.05$. For comparison of categorical variables between groups, the Chi-square test was used at 95% confidence interval.

RESULTS

The study analyzed staining intensities in different lesion types focusing on Hematoxylin and Eosin (H&E) and Periodic Acid-Schiff (PAS) stains. The results showed significant differences in mean staining intensities across the study groups. The dysplasia group had higher mean H&E and PAS staining scores compared to the Oral Squamous Cell Carcinoma (OSCC) group. The control group exhibited the highest staining intensities for both H&E and PAS among all groups. These findings are illustrated in Figure 1.

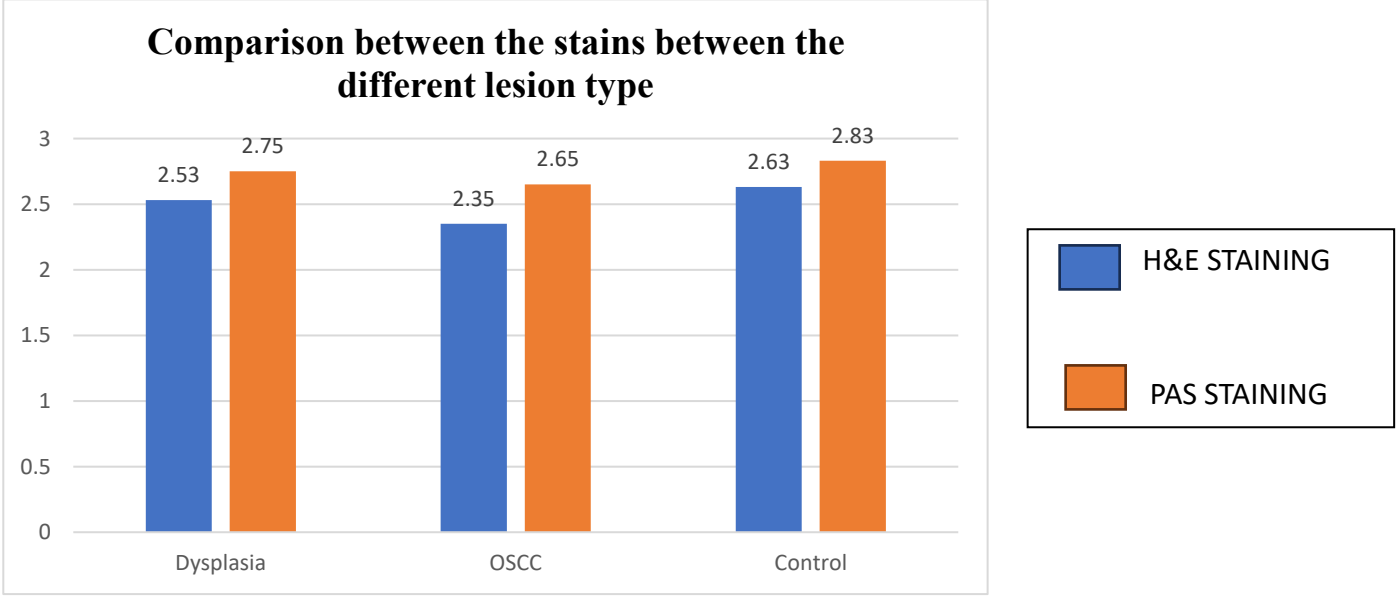

Figure 1: Graphical representation of the comparison between the different lesion type without Alcian Blue

Post-hoc tests using Turkey HSD confirmed significant differences in mean staining intensities between the dysplasia and OSCC groups, as well as between the OSCC and control groups, for both H&E and PAS stains, indicating distinct staining patterns.

For H&E staining, severity scores were examined by lesion type and grade. In the dysplasia group, severe dysplasia had the highest mean H&E staining intensity, followed by mild and moderate dysplasia. In the OSCC group, well-differentiated OSCC showed the highest mean H&E intensity, followed by poorly differentiated and moderately differentiated OSCC. These differences within subgroups were not statistically significant, as shown in Figure 2.

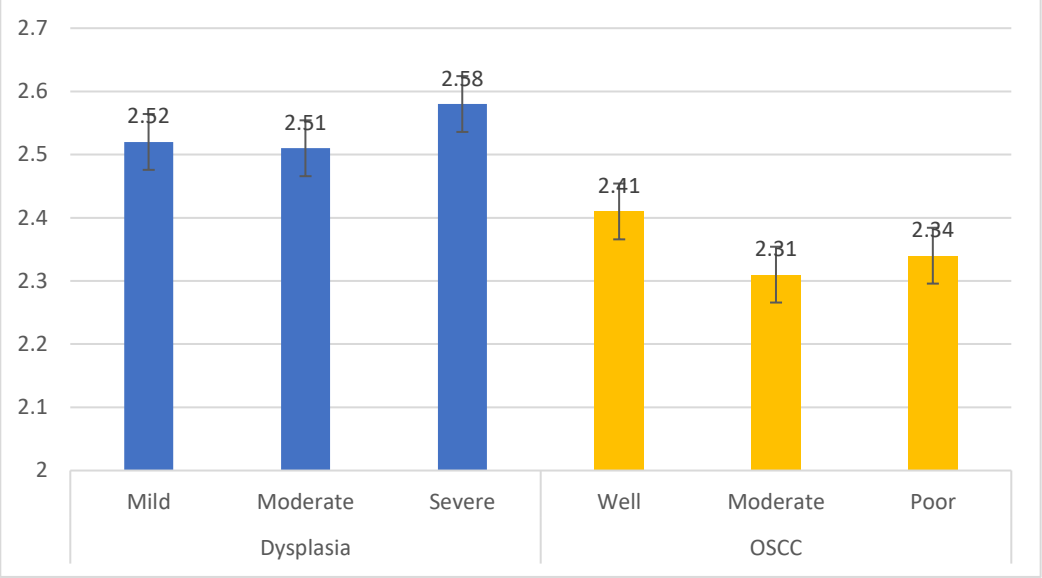

Figure 2: Graphical representation of the comparison of the type severity of the lesion for H&E staining

For PAS staining, severity scores varied by lesion type and grade. In the dysplasia group, mild dysplasia had the highest mean PAS staining intensity, followed by moderate and severe dysplasia. In the OSCC group, well-differentiated OSCC had the highest mean PAS intensity, followed by moderately differentiated and poorly differentiated OSCC. These differences within both dysplasia and OSCC subgroups were statistically significant, as depicted in Figure 3.

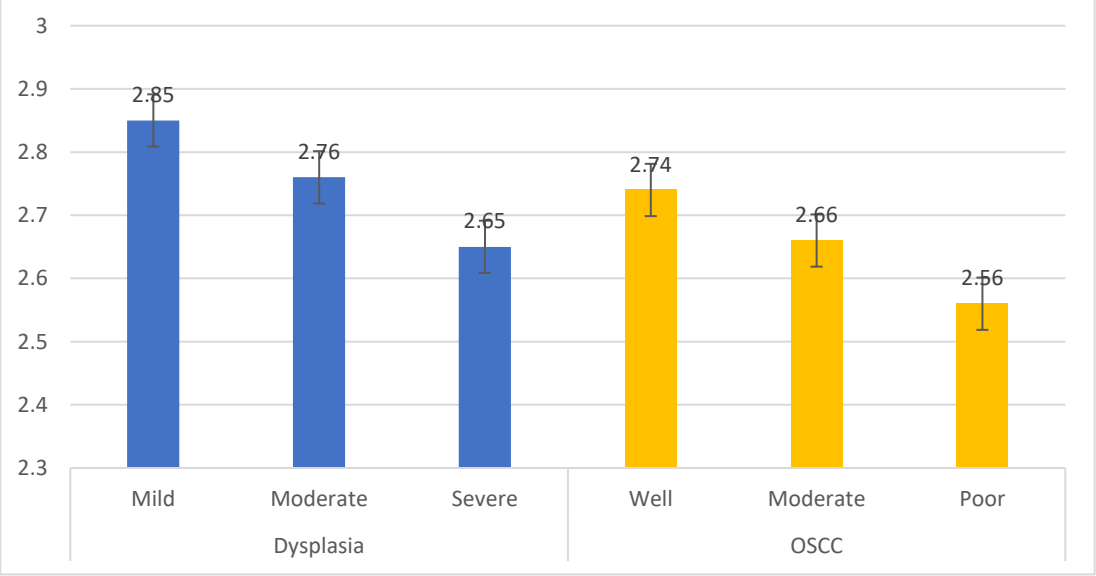

Figure 3: Graphical representation of the comparison of the type severity of the lesion for PAS staining

DISCUSSION

The extracellular matrix (ECM) is a dense latticework of collagen and elastic fibres which is embedded as a ground substance and is composed of collagen, elastin, reticulin, fibronectin, laminin, proteoglycans, glycoproteins and glycosaminoglycans. This extracellular matrix acts as a scaffold which isolates tissue compartments, mediates cell attachment and influences tissue architecture with both structural and functional characteristics. Interactions between normal cells and the matrix may be altered in neoplastic lesions and thus may influence tumour proliferation and invasion.

In the present study, histochemical analysis using Periodic Acid-Schiff (PAS) staining revealed a predominantly weak and diffuse staining pattern across progressive grades of Oral Epithelial Dysplasia (OED). Specifically, mild, moderate, and severe dysplasia exhibited weak to minimal PAS staining intensity, indicating a gradual reduction in mucin content, particularly within the juxta- epithelial connective tissue region. This downward trend in mucin expression from mild to severe OED may reflect the progressive loss of normal epithelial function and the onset of neoplastic transformation. Importantly, in OED, dysplastic changes remain confined to the epithelial compartment without invasion beyond the basement membrane (BM). As a

result, the BM remains intact and demonstrates prominent PAS positivity, likely due to the preserved presence of glycoprotein-rich structural components such as laminin, entactin, and heparan sulfate proteoglycans, which are known PAS-reactive elements.

In cases of Oral Squamous Cell Carcinoma (OSCC), PAS staining demonstrated predominantly weak diffuse positivity across most histological grades. However, in poorly differentiated OSCC, the staining pattern shifted, showing focal PAS positivity. This reduction and localization in staining intensity may correspond to significant alterations in glycoprotein expression and distribution associated with increased cellular dedifferentiation and tumour aggressiveness. Epithelial-derived malignancies, including OSCC, are known to produce detectable levels of glycoproteins, which can serve as potential diagnostic markers.

The observed weak glycoprotein reactivity within the stromal compartment of OSCC may be attributable to proteolytic degradation and remodelling of the extracellular matrix—a hallmark of invasive carcinoma. Tumour cell invasion often necessitates enzymatic breakdown of structural stromal components, facilitating the migration and dissemination of dysplastic epithelial cells. Matrix metalloproteinases (MMPs) and other proteases secreted by tumour cells and stromal fibroblasts can degrade mucin-associated glycoproteins, contributing to the reduced PAS positivity observed in the stroma. These findings support the hypothesis that mucin degradation and ECM remodelling are integral to the invasive behaviour of OSCC and may offer valuable histopathological indicators of tumour progression.[7]

OSCC is characterized by pearl shaped islands of malignant epithelial cells in the connective tissue. These tumour cells possess the capability to secrete proteins that may mimic the biochemical composition of the native basement membrane (BM). This phenomenon could explain the distinct PAS-positive staining observed selectively around tumour islands in OSCC, likely attributable to reduplicated or newly synthesized basement membrane-like material secreted by neoplastic epithelial cells. Quantitative and qualitative alterations of the BM during carcinogenesis are well-documented and play a pivotal role in the processes of tumour invasion and metastasis.[7] These changes facilitate the degradation of structural barriers, enhance cellular motility, and enable neoplastic cells to penetrate the surrounding connective tissue.

Besides its affinity for glycoproteins, the Periodic Acid–Schiff (PAS) staining technique is known to highlight other BM-related and carbohydrate-rich structures, including glycogen, starch, and various basement membrane constituents present within the connective tissue matrix.[8] PAS staining is particularly sensitive to neutral mucins, as well as acid mucins—both simple non-sulfated and complex sulfated forms.[9] However, it is important to note that PAS does not stain acid simple mesenchymal mucins or acid complex mucins associated with connective tissue.[9]

In our study, it is been observed that for H&E stain intensity staining Severe dysplasia had the highest mean score followed by mild and moderate dysplasia in OED group. In OSCC group Well differentiated OSCC had the highest mean score followed by poorly differentiated and moderately differentiated.

For PAS staining in OED group Mild dysplasia had the highest mean score followed by Moderate dysplasia followed by severe dysplasia. In the OSCC group, highest mean score for PAS stain intensity was noted for well differentiated OSCC, followed by moderate and lowest for poor.

It was seen that when overall lesion mean scores were compared, a statistically significant difference was noted between the groups. Higher mean score for both the stain was noted for the dysplasia group as compared to the OSCC group. PAS Stain shows high intensity in all group.

The present study provides a comprehensive histochemical analysis of mucin expression and extracellular matrix (ECM) remodelling during the progression from oral epithelial dysplasia (OED) to oral squamous cell carcinoma (OSCC), utilizing Periodic Acid–Schiff (PAS) and Haematoxylin and Eosin (H&E) staining techniques. The findings reveal significant alterations in mucin profiles and staining patterns across histological grades, offering insights into the molecular and structural dynamics of oral carcinogenesis.

Mucin Expression and Tumour Progression

The observed increase in neutral mucin staining intensity from OED to OSCC, particularly in the juxta- epithelial connective tissue and surrounding tumour islands, underscores the role of mucins in creating a tumour-supportive microenvironment.[10] Neutral mucins, detected by PAS staining, are likely secreted by epithelial tumour cells or reactive stromal cells, as suggested by George J. et al.[10.] This increase may enhance cellular adhesion, modulate signalling pathways, and facilitate immune evasion, all of which are critical for tumour progression. The prominence of neutral mucins in OSCC aligns with their role in stabilizing tumour foci and supporting invasive behaviour, as they may act as a scaffold or protective barrier.

Conversely, the progressive decline in acid mucin staining from mild to severe OED, with minimal to absent staining in severe dysplasia, reflects the loss of normal epithelial function during neoplastic transformation. The weak acid mucin positivity observed around tumour islands in OSCC, with no significant variation across histological grades, suggests that acid mucins play a limited role in advanced malignancy. The shift from mixed (sulfated and carboxylated) mucins in early OED to predominantly sulfated mucins in poorly differentiated OSCC indicates a biochemical transition that may be linked to increased tumour aggressiveness. This finding is consistent with George J. et al.[10], who reported similar mucin transitions in OSCC, highlighting the potential of sulfated mucins as markers of advanced disease.

PAS and H&E Staining Patterns

The PAS staining results reveal a distinct pattern of mucin and glycoprotein distribution. In OED, the gradual reduction in PAS staining intensity from mild to severe dysplasia corresponds to the progressive loss of mucin content, particularly in the juxtaepithelial region. The intact basement membrane (BM) in OED, which exhibited prominent PAS positivity, likely reflects preserved glycoprotein-rich components such as laminin, entactin, and heparan sulfate proteoglycans. These components are known to be PAS-reactive and are critical for maintaining epithelial integrity. In contrast, OSCC displayed weak diffuse PAS positivity across most grades, with focal positivity in poorly differentiated cases. This shift may be attributed to ECM remodelling and proteolytic degradation by matrix metalloproteinases (MMPs) and other proteases, which facilitate tumour invasion by breaking down structural stromal components.

The H&E staining results further complement these findings. In OED, the highest staining intensity in severe dysplasia reflects increased cytological atypia and architectural disorganization, hallmarks of early neoplastic change. In OSCC, well-differentiated cases showed the highest H&E staining intensity, consistent with their retained epithelial characteristics, while poorly differentiated cases exhibited lower intensity, indicative of dedifferentiation. The statistically significant difference in mean staining scores between OED and OSCC groups underscores the distinct histopathological features of these conditions, with higher scores in OED reflecting less invasive behaviour compared to OSCC.

ECM Remodelling and Tumour Invasion

The weak PAS positivity in the stromal compartment of OSCC highlights the role of ECM remodelling in tumour invasion. Proteolytic degradation of mucin-associated glycoproteins by MMPs and other proteases, secreted by both tumour cells and stromal fibroblasts, facilitates the migration and dissemination of dysplastic epithelial cells. This process is a hallmark of invasive carcinoma and is supported by the observed reduction in stromal glycoprotein reactivity.[11] Additionally, the distinct PAS-positive staining around tumour islands in OSCC suggests the secretion of BM-like material by neoplastic epithelial cells. This phenomenon, often described as BM reduplication, may mimic the biochemical composition of the native BM, enabling tumour cells to breach structural barriers and enhance motility. These findings align with the literature, which documents quantitative and qualitative BM alterations as pivotal events in tumour invasion and metastasis.[12,13]

Clinical and Diagnostic Implications

The differential staining patterns of mucins and glycoproteins across OED and OSCC grades offer potential diagnostic and prognostic value. The progressive increase in neutral mucin

expression and the shift to sulfated mucins in advanced OSCC could serve as histopathological indicators of tumour progression and aggressiveness. The preserved PAS positivity of the BM in OED, contrasted with its alteration in OSCC, may aid in distinguishing pre-invasive from invasive lesions. Furthermore, the weak stromal PAS positivity in OSCC could reflect the extent of ECM degradation, providing a marker for invasive potential. These histochemical markers, combined with H&E staining, enhance the ability to characterize the histopathological spectrum of oral carcinogenesis.

Limitations and Future Directions

While this study provides valuable insights, certain limitations warrant consideration. The lack of prior literature on acid mucin histochemistry in OED highlights a gap in understanding their specific role in oral precancerous lesions. Additionally, the study focused on PAS and H&E staining, which, while effective, may not capture the full spectrum of molecular changes in mucin glycosylation or ECM composition. Future studies could employ advanced techniques such as immunohistochemistry or mass spectrometry to explore specific mucin subtypes (e.g., MUC1, MUC4) and their glycosylation patterns. Investigating the role of specific proteases, such as MMP-2 and MMP-9, in ECM remodelling could further elucidate the mechanisms driving mucin degradation. Moreover, longitudinal studies tracking mucin changes in patients with OED progressing to OSCC could validate these findings and establish their prognostic significance.

CONCLUSION

This study highlights the dynamic changes in mucin expression and ECM remodelling during the progression from OED to OSCC. The increase in neutral mucins, decline in acid mucins, and shift to sulfated mucins reflect the evolving tumour microenvironment and its role in facilitating invasion and metastasis.[14] PAS and H&E staining provide complementary insights into these histopathological changes, with potential applications as diagnostic markers. These findings contribute to the growing body of evidence on mucin histochemistry in oral carcinogenesis and underscore the need for further research to translate these observations into clinical practice.

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