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# Examining Connective Tissue Changes Across Various Grades of Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma: A Histochemical Polymeric Investigation

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***Abstract***

*Oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) represent a spectrum of potentially malignant disorders posing significant challenges in diagnosis and management. The tumor microenvironment, particularly alterations in the connective tissue, plays a crucial role in the progression of these lesions. This histochemical investigation comprised with embedded polymers aimed to examine connective tissue changes across various grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC). OED and OSCC represent a spectrum of potentially malignant disorders of the oral mucosa, with varying degrees of cellular and tissue alterations. However, the specific changes occurring in the connective tissue microenvironment across different grades of OED and OSCC remain poorly understood. In this study, histological sections from biopsied specimens of OED and OSCC were subjected to histochemical analysis to assess alterations in connective tissue components such as collagen fibers, mucins, and vascularization. Results revealed distinct patterns of connective tissue changes associated with different grades of OED and OSCC, highlighting the potential diagnostic and prognostic significance of these alterations in oral cancer progression through nanotechnological polymeric investigation.*

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## INTRODUCTION

Oral cancer represents a significant global health concern.[1] And we are at the epicentre of this global disaster with approximately two-thirds of patients from Southeast Asia [2]. Cancer biology is complex and the better we understand it, the more accurate will be its management. OSCC is an epithelial tumour that exhibits mutated epithelial cells with varying degrees of squamous differentiation. By breaking through the basement membrane, these mutant cells enter the connective tissue stroma, causing the stroma to change dynamically [3].

The tumour-suppressing properties of connective tissue extracellular matrix (ECM) help to maintain healthy epithelial tissues, and they also work in concert with cancer cells to control the course of the

 

disease. The extracellular matrix (ECM) is a constantly evolving macromolecular structure responsible for regulating cellular movement, proliferation, and differentiation. It consists of three fundamental components: fibrous structural proteins, glycoproteins, and proteoglycans [4]. Type IV collagen, a structural fibrous protein, undergoes significant degradation during cancer progression, primarily regulated by the secretion of metalloproteinases (MMP) enzymes by neoplastic cells. This degradation potential typically escalates during advanced stages of invasion [5]. In cancer, neoplastic cells infiltrate the underlying connective tissue, leading to degradation of the extracellular matrix (ECM). This process triggers the release of cytokines and growth factors, such as transforming growth factor-beta (TGF-β), which in turn promote the activation and progression of malignancy [6,7].

Regressive changes in glycoproteins and glycosaminoglycans are observed, which are also mediated by MMPs, resulting in the weakening of stromal components, allowing tumour cells to proliferate and migrate. As a result, they serve as a meshwork for tumour cell proliferation. Lysis also causes the release of cytokines and growth factors, which promote tumour cell growth [8-9].

Analysing the complex nature of the extracellular matrix, rather than just the heterogeneities of tumour cells, will result in more robust management of OSCC based on improved understanding of tumour progression and metastasis. Integrating these concepts into a prognostic framework could facilitate the prediction of tumour behaviour and enhance clinical outcomes.[10]

Therefore, the objective of our study is to establish a correlation between tumour biological behaviour (grading) by assessing extracellular matrix alterations and nanotechnological advancement across various histopathological grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) utilizing specific histochemical polymeric stains.

## REVIEW LITERATURE

Oral Potentially Malignant Disorders (OPMD) is a classification introduced by the WHO in 2017 to denote lesions or conditions with an increased propensity for malignant progression. These conditions are histo pathologically categorized as mild, moderate, or severe OED. It is noted that OSCC is more likely to arise from severe OED.

Neoplastic epithelial cells and the extracellular matrix within which they are spread are the two distinct parts of OSCC [11]. The epithelium undergoes a variety of changes, including the breakdown of the basal lamina, as OSCC develops. Additionally, it is known that the extracellular matrix serves as a functional component of OSCC in addition to providing structural support. The tumours' ability to grow and receive nutrition depends on extracellular matrix components. The matrix also serves as a defense system against malignant tumours and hence can be a double edge sword. Proteolysis of the extracellular matrix regulates the migration of normal cells. In cancer, proteolytic re-modelling of the extracellular matrix components promotes tumour progression by facilitating the invasion of tumour cells. This is followed by changes in the collagenous stroma. By regulating the growth factor, the coordinated activity of mesenchymal and epithelial cells promotes the growth of epithelial cells.

Though we know that tumour microenvironment influences OED and OSCC, there are still lacunae in understanding how different stages of neoplasia are affected by fibrous elements (such as collagen, reticulin fibres and elastin) and ground substances (such as mucins, glycoproteins and fibrin).Oral epithelial dysplasia (OED) is frequently a precursor to OSCC, WHO in the year 2017 classified these pathologies as oral potentially malignant disorders (OPMD) and gave a new classification system which employs cytological and architectural features to achieve a more objective stance to OED diagnosis and grading [12].

Jaber MA *et al*. [13] in the year 2020 did a long-term study of 359 patients with OED, 5.5% of cases in their study developed oral squamous cell carcinoma with a mean transformation time of 3.3 years.

 

The average age at the onset of OSCC diagnosis was 52.6 years, with 55% of patients being male. Additionally, it is noteworthy that only 55% of OSCC patients are aged 50 years or older.

Krolls and Hoffman [14] In 1976, they conducted an extensive examination of oral squamous cell carcinoma, meticulously analyzing a total of 1,449 cases utilizing AIFP files. Their findings revealed that 38% of cases were in the lower lip, followed by 22% and 17% in the tongue and floor of the mouth, respectively. 31.8% of the population was in the $7^{th}$ decade and 86.8% of cases were seen between the $4^{th}$ and $8^{th}$ decade. 93% of cases were recorded in males. Compared to the black race (5.2%), the white race (92.6%) had a higher predisposition.

Petti S *et al*. [15] found that the mutagenic effects of all these products depend on the frequency and duration of use, whereas using multiple such products simultaneously has an outsized impact. However, apart from these carcinogenic agents, another etiopathogenesis of OSCC has recently been implicated, including human papillomavirus (HPV) infection and a diet that is low in vegetables and fruits.

According to Suvarna KS *et al. [16]*, once the sections are made, excess paraffin wax were cleared with two changes of xylene then these tissue sections were hydrated with water and Verhoeff's staining will be done for 15 - 25 minutes. After staining the sections were washed with tap water, differentiated in 2% of aqueous ferric chloride and once again washed in water. Rinsing with 95% alcohol may help to remove excess iodine from the sections. Followed by this staining with van Gieson or eosin stain was done and dehydrated with 2 changes of alcohol. Before mounting, the sections were cleared in xylene. As an outcome, the elastic fibre-stained black colour and depending on the counter stain the other tissue will stain accordingly.

Patankar SR *et al*. [17] in the year 2016 found that although these fibres are insoluble, they can be degraded or destroyed in some instances, such as in tumours, by specific proteases, elastases and matrix metalloproteinases (MMPs) secreted by tumour cells. Patankar SR et al. [17] investigated collagen fibres in 2016 using the Van Gieson stain and picrosirius red stain and determined that type 1 collagen fibres are seen in cases of well and moderately distinct OSCC and type 3 collagen fibres in cases of poorly distinct OSCC. According to the findings, well-differentiated OSCC had an abundance of thick collagen fibres, moderately differentiated OSCC had dispersed collagen fibres, and poorly differentiated OSCC had thin few dispersed collagen fibres.

Mehregan AH *et al*. [18] examined elastic fibres in pigmented nevi in 1962 and observed that the amount and pattern of elastic fibres tend to be uniform in all nevi excised from various sites of the same individual. The vast majority of tumours have hyperplasia of the fibres present in between and around the deepest nests of nevus cells. The elastic fibres eventually became finer and thinner in the superficial part of the tumour. The network of hyperplastic fibres was evident in the deeper section between the nests of benign nevus cells but was absent in the superficial sections.

Mehregan AH *et al*. [19] examined elastic fibres in basal cell epithelioma in 1964 and classified 5 different morphological patterns based on their frequency. The elastic fibres are absent in 60% of incidences, degenerated elastic fibres were found in 17.2% of incidents, normal fibres in 9.4% of cases, an increase in the density of elastic fibres at the corium in 8.8% of cases, and newly formed elastic fibres in 4.6% of cases. Elasbali AM et al. [20] analyzed the reticulin fibre shape in breast lesions in 2018 and revealed that strong grades of reticulin fibre staining were attained with cancer tissues. With fibrocystic abnormalities, reticulin fibre staining grades were reduced. Breast cancer has considerably more reticulin fibres than benign breast tumours ($P < 0.05$).

Agrawal DN et al. [21] conducted a study on mucins found in prostatic lesions in 2014 to discriminate malignant from benign lesions based on the presence and composition of neutral or acidic mucin. Only neutral mucins were found in benign hyperplasia of the prostate, whereas both neutral and acidic mucins

 

were found in prostate carcinoma. When acidic mucins in prostatic cancer and benign hyperplasia were compared, it was statistically significant (P = 0.001) with more in prostate cancer.

According to Velidandla S [22] staining the normal buccal mucosa with picrosirius red stain reveals greenish yellow birefringence in most of the fields adjacent to the basement membrane, blood vessels and around the muscles, whereas deeper sections of lamina propria reveal reddish-orange or yellowish birefringence in most of the fields.

Sharma R et al. [23] analyzed collagen fibres using a picrosirius red stain in 2015 and observed that the colour of these fibres changed from yellowish orange to yellow greenish as the grade progressed from mild to moderate OED. The collagen fibres turn to a greenish hue as the grades advance from moderate to severe dysplasia.

## MATERIALS AND METHODS;

The current investigation was carried out at the Department of Oral Pathology and Microbiology, within the School of Dental Sciences at Krishna Institute of Medical Sciences Deemed to Be University (KIMSDTBU), located in Karad. Tissue blocks were obtained from the records of the Department of Oral Pathology, also situated within the School of Dental Sciences at KIMSDTBU in Karad, for subsequent analysis.

### Inclusion criteria

Individuals with histologically confirmed cases of Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma were included in the study.

### Exclusion criteria

Blocks containing insufficient tissue samples were excluded from the study to ensure the adequacy of specimens for analysis. Additionally, cases diagnosed as carcinoma in-situ or micro-invasive OSCC were excluded, as were cases lacking grading information. Variants of OSCC were also excluded from the sample to maintain homogeneity and consistency within the study cohort. These criteria were implemented to enhance the accuracy and reliability of the research findings by focusing on cases meeting predefined standards for inclusion.

### Study subjects

The study material consisted of 90 formalin-fixed, paraffin-embedded tissue blocks, which were divided into six groups: Group I, Group II, Group III, Group IV, Group V, and Group VI.

The study samples were divided into individual groups based on standard classifications. The three grades system for OED by WHO (2017)[78] classification was adopted to classify OED as mild, moderate or severe dysplasia. In this study, the most commonly used Broder's (1927)[78] classification system was used to classify OSCC as well, moderate or poorly differentiated squamous cell carcinoma.

***Group I:*** 15 cases of histo pathologically confirmed mild OED tissue specimens.
***Group II:*** 15 cases of histo pathologically confirmed moderate OED tissue specimens.
***Group III:*** 15 cases of histo pathologically confirmed severe OED tissue specimens.
***Group IV:*** 15 cases of histo pathologically confirmed well differentiated OSCC tissue specimens.
***Group V:*** 15 cases of histo pathologically confirmed moderately differentiated OSCC tissue specimens.
***Group VI:*** 15 cases of histo pathologically confirmed poorly differentiated OSCC tissue specimens.

### Materials and Equipment

For the research study, various materials and equipment were utilized to process and analyze tissue specimens from 45 cases of oral epithelial dysplasia (OED) and 45 cases of oral squamous cell

 

carcinoma (OSCC), with 15 cases per grade for each condition. The tissue specimens were preserved using formalin fixation and paraffin embedding methods. The equipment included a microtome (Leica RM-2125RTS) and microtome blades (Leica 818) for tissue sectioning, slides, a hot plate/slide warmer, measuring jars, and a weighing machine. Glassware and essentials required for staining procedures were also utilized, along with an electronic timer and Coplin jars for staining processes. Clearing agent xylene, alcohol, and distilled water were used in tissue processing steps. Various stains were employed for histological evaluation, including Hematoxylin & Eosin stain for grading, Verhoeff-van Gieson stain for elastic fibers, Modified Gomori's stain for reticulin fibers, Picrosirius red stain for collagen fibers, Alcian blue – Periodic acid Schiff stain to differentiate acid mucins from neutral mucins, and Aldehyde fuchsin – Alcian blue to differentiate sulphated mucins from acid mucins. DPX mountant and coverslips were used for slide mounting. The examination of stained tissue sections was conducted using a binocular microscope (MAGNUS – OLYMPUS, Theia) and a polarized microscope (OLYMPUS UPRIGHT MICROSCOPE BX43), with a camera utilized for photomicrography purposes. These materials and equipment were crucial for conducting thorough histological evaluations and analyses as part of the research study.

**Methodology**

The comprehensive sample comprising 45 cases each of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) was obtained from the archival materials of the Department of Oral Pathology and Microbiology. The selection process involved assessing Hematoxylin and Eosin (H&E) slides from these cases based on predefined inclusion and exclusion criteria. Demographic details and biopsy site information were retrieved from existing institutional records. Subsequently, 6-micron thick sections were obtained from the selected paraffin-embedded blocks using a rotary microtome, and sets of five slides were prepared. These tissue sections were then transferred onto albumin-coated slides using a tissue floatation bath to create ribbons of tissue sections. Each slide was meticulously labeled with the corresponding biopsy numbers to ensure easy identification for future reference. Following preparation, the slides underwent staining with five different stains, including Verhoeff-van Gieson stain, Modified Gomori's stain, Picrosirius red stain, Alcian blue – Periodic acid Schiff stain, and Aldehyde fuchsin – Alcian blue, enabling comprehensive histological evaluation and analysis.

## OBSERVATIONS AND RESULTS

It was found that the 46-60 age group exhibited the highest percentage of cases in OED, while the predominant age group impacted by OSCC was between 61 and 90 years old. Despite fewer reported cases in the 25-45 age group, the percentage observed in both OED and OSCC remains concerning, indicating the importance of early detection and prevention measures across all age ranges Shown in Table 1.

**Table 1.** Distribution of Age Based on Diverse Stages of OED and OSCC.

| **Grades** | **Age group in years and number of patients.** | | |
|---|---|---|---|
| | **25 – 45 Number (%)** | **46 – 60 Number (%)** | **61 – 90 Number (%)** |
| Mild OED | 5 (33.3) | 9 (60) | 1 (6.7) |
| Moderate OED | 3 (20) | 6 (40) | 6 (40) |
| Severe OED | 3 (20) | 8 (53.3) | 4 (26.7) |
| Well differentiated OSCC | 3 (20) | 6 (40) | 6 (40) |
| Moderately differentiated OSCC | 4 (26.7) | 6 (40) | 5 (33.3) |
| Poorly differentiated OSCC | 7 (46.7) | 2 (13.3) | 6 (40) |

All cases of OED and OSCC showed a predilection for the male gender depicted in Table 2.

 

**Table 2.** Distribution of Patients Based on Sex in Diverse Stage of OED and OSCC.

| Grades | Sex wise distribution and number of patients | |
|---|---|---|
| | Male Number (%) | Female Number (%) |
| Mild OED | 11 (73.3) | 4 (26.7) |
| Moderate OED | 13 (86.7) | 2 (13.3) |
| Severe OED | 12 (80) | 3 (20) |
| Well differentiated OSCC | 10 (66.7) | 5 (33.3) |
| Moderately differentiated OSCC | 13 (86.7) | 2 (13.3) |
| Poorly differentiated OSCC | 12 (80) | 3 (20) |

**Changes in elastic fibres;**

***Changes in density of elastic fibres:***

The analysis from Figure 1 revealed distinct patterns in the density of elastic fibers across diverse stages of OED and OSCC. Among the 14 cases of mild OED, 93.3% exhibited moderate density of elastic fibers, while one case (6.7%) showed abundant fibers in the connective tissue stroma. In moderate OED cases (86.7%), moderate fiber density was predominant, with a minority (13.3%) showing scanty fibers. Conversely, all severe OED cases demonstrated scanty elastic fiber density. For well-differentiated OSCC (80% of cases), moderate fiber density prevailed, with abundant fibers observed in 20% of cases. In moderately differentiated OSCC (66.7% of cases), abundant fibers were more common, while 33.3% showed moderate density. Poorly differentiated OSCC cases (93.3%) predominantly exhibited scanty fibers, with a single case (6.7%) displaying moderate density. Importantly, a highly notable relationship ($p<0.001$) was identified between the various stages of OED and OSCC and the density of elastic fibers, underlining the potential diagnostic value of this association. When Spearman correlation was used it was found that there was a strong negative relation (-0.844) was noted for OED and moderate negative correlation (-0.604) was noted for OSCC. This may be interpreted as; the density of elastic fibres decreases with increasing grade of OED and OSCC.

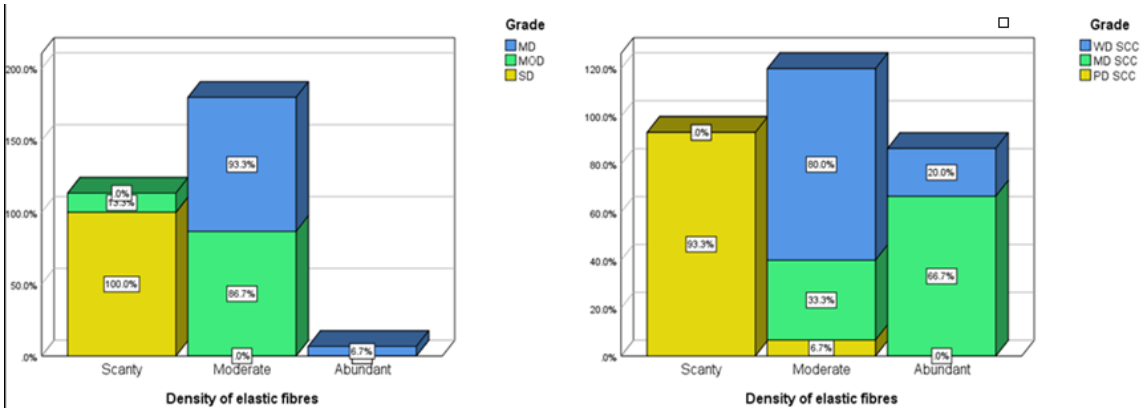


**Figure 1.** *Correlation of Elastic Fibre Density in Diverse Stage of OED and OSCC.*

***Changes in length and thickness of elastic fibre***

The analysis from Figure 2 of elastic fiber density across diverse stages of OED and OSCC revealed distinct patterns. Among mild OED cases, 93.3% showed moderate fiber density, with a single case (6.7%) displaying abundant fibers. Moderate OED cases (86.7%) predominantly exhibited moderate fiber density, while all severe OED cases displayed scanty elastic fiber density. In well-differentiated OSCC cases (80%), moderate fiber density was prevalent, whereas 20% showed abundant fibers. Moderately differentiated OSCC cases (66.7%) commonly displayed abundant fibers, while 33.3% exhibited moderate fiber density. Poorly differentiated OSCC cases (93.3%) predominantly showed scanty fibers, with only one case (6.7%) displaying moderate density. Notably, a highly significant relationship ($p<0.001$) was noted among the diverse stage of OED and OSCC and the density of elastic fibers, suggesting the potential diagnostic importance of this association.

 

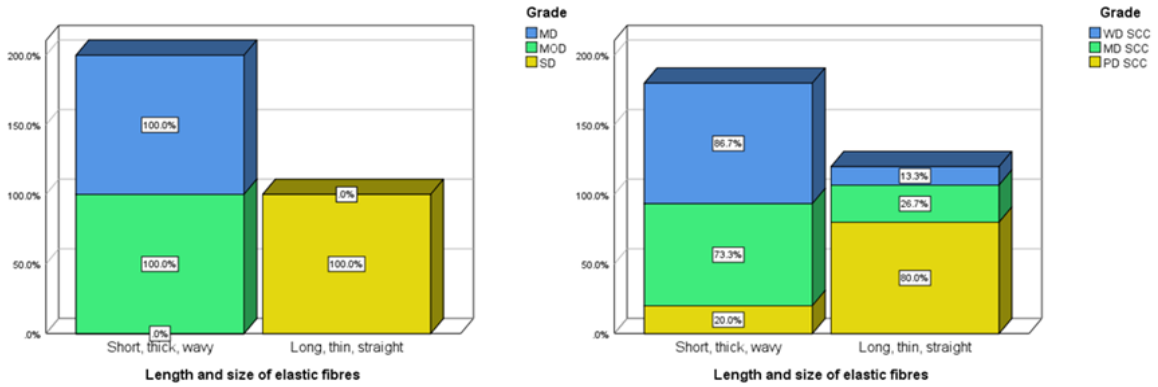


**Figure 2.** Correlation of Elastic Fibres Length and Thickness in Diverse Stages of OED and OSCC

***Changes in orientation of elastic fibres***

The examination of elastic fiber arrangements in relation to the overlying epithelium revealed distinct patterns across diverse stages of OED and OSCC. In mild and moderate OED cases, parallel arrangements of elastic fibers with the overlying epithelium were consistently observed. Conversely, severe OED cases consistently exhibited perpendicular arrangements of elastic fibers to the overlying epithelium. Among well-differentiated (WD) OSCC cases, 80% displayed parallel arrangements and 20% showed perpendicular arrangements of elastic fibers. For moderately differentiated (MD) OSCC cases, 73.3% demonstrated parallel arrangements, while 26.7% displayed perpendicular arrangements. In poorly differentiated (PD) OSCC cases, 73.3% exhibited perpendicular arrangements, and 26.7% showed parallel arrangements of elastic fibers. Statistical analysis revealed a notable correlation between the grades of OED ($p<0.001$) and OSCC ($p<0.05$) and the orientation of elastic fibers to the overlying epithelium. Spearman correlation further indicated a strong correlation (0.866) in OED and a moderate correlation (0.444) in OSCC among the various grades of lesions with the orientation of elastic fibers to the overlying epithelium, suggesting that as the grade increases, the orientation of elastic fibers tends to become perpendicular to the overlying epithelium shown in Table 3.

**Table 3.** Correlation of Elastic Fibres Orientation to Overlying Epithelium in Diverse Stage of OED and OSCC

| **Oral Epithelial Dysplasia** | | **Grade** | | | **Total** | **Spearman correlation** | |
|---|---|---|---|---|---|---|---|
| | | **Mild** | **Moderate** | **Severe** | | **r** | **p** |
| Orientation of elastic fibres to overlying epithelium | Parallel % | 15 | 15 | 0 | 30 | 0.866 | (<0.001)** |
| | | 100.0 | 100.0 | 0.0 | 66.7 | | |
| | Perpendicular % | 0 | 0 | 15 | 15 | | |
| | | 0.0 | 0.0 | 100.0 | 33.3 | | |
| Total% | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |
| **OSCC** | | **Grade** | | | **Total** | **r** | **p** |
| | | **WD** | **MD** | **PD** | | | |
| Orientation of elastic fibres to overlying epithelium | Parallel % | 12 | 11 | 4 | 27 | 0.444 | (0.002) * |
| | | 80.0 | 73.3 | 26.7 | 60.0 | | |
| | Perpendicular % | 3 | 4 | 11 | 18 | | |
| | | 20.0 | 26.7 | 73.3 | 40.0 | | |
| Total % | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |

*r represents the Spearman correlation coefficient itself, which quantifies the strength and direction of the monotonic relationship between two variables.*
*p is the p-value associated with the Spearman correlation coefficient. This value assesses the statistical significance of the observed correlation.*

 

**Changes in reticulin fibres;**

***Changes in length of reticulin fibres***

In the examination of reticulin fibers, it was found in Fig. 3 that they were absent in all cases of mild and moderate Oral Epithelial Dysplasia (OED), with only 13.3% of severe OED cases showing long reticulin fibers. Among well-differentiated Oral Squamous Cell Carcinoma (OSCC) cases, 73.3% displayed long reticulin fibers, while 26.7% had short fibers. In moderately differentiated OSCC cases, 66.7% exhibited short reticulin fibers, whereas 33.3% showed long fibers. For poorly differentiated OSCC, 60% of cases had very short reticulin fibers, while 20% of well and moderately-differentiated OSCC cases showed long and short reticulin fibers, respectively. A highly notable relationship ($p<0.001$) was identified between the various grades of OSCC and the length of reticulin fibers. Spearman correlation analysis revealed a weak correlation (0.23) in OED cases and a moderate correlation (0.589) in OSCC cases when comparing the various grades of lesions with the length of reticulin fibers, indicating that as the grade increases, the length of reticulin fibers tends to decrease.

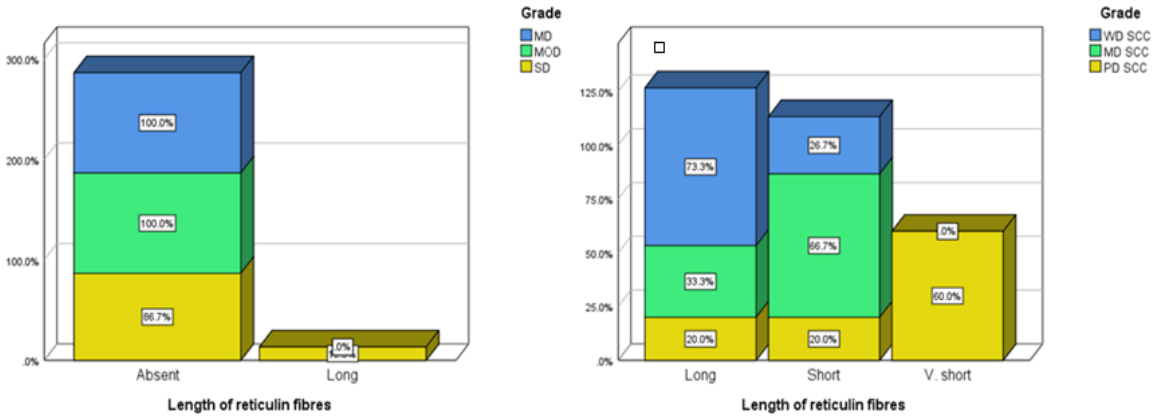


**Figure 3.** Correlation of Length of Reticulin Fibres in the Diverse Stage of OED and OSCC

***Changes in density of reticulin fibres***

In the assessment of reticulin fibers, it was found in Fig. 4 that they were consistently absent in all cases of mild and moderate OED, while only 13.3% of severe OED cases exhibited scanty density. Abundant density of reticulin fibers was observed in 73.3% of well-differentiated OSCC cases, 46.7% of moderately differentiated OSCC cases, and 73.3% of poorly differentiated OSCC cases. However, no notable relationship was identified between the various grades of OED and OSCC and the density of reticulin fibers, despite the observation that higher stages of both OED and OSCC tended to have more abundant reticulin fibers.

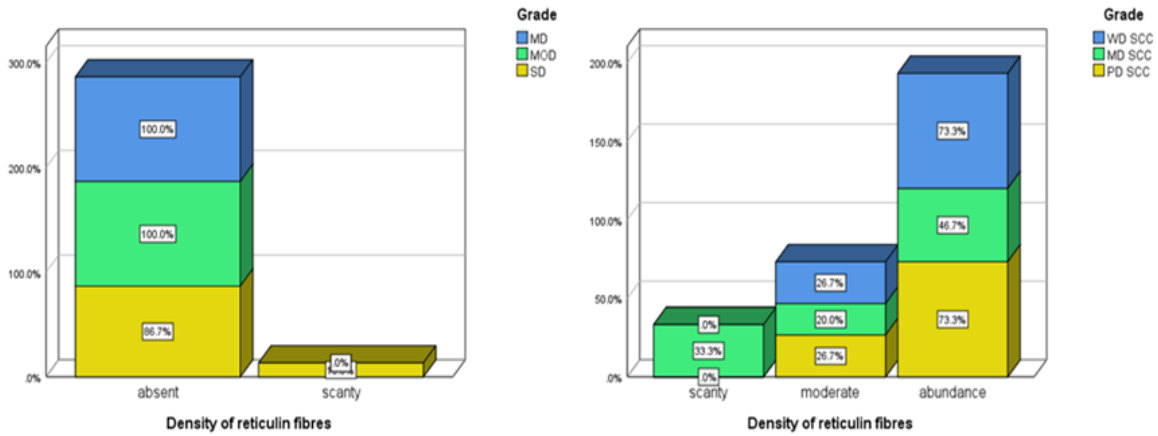


**Figure 4.** Correlation of Density of Reticulin Fibres in Diverse Stages of OED and OSCC

 

***Changes in staining intensity of reticulin fibres***

Staining intensity analysis revealed that in severe Oral Epithelial Dysplasia (OED), strong staining was evident in 13.3% of cases. Among well-differentiated (WD) Oral Squamous Cell Carcinoma (OSCC) cases, 60% exhibited strong staining intensity, while 40% showed weak staining. Similarly, in moderately differentiated (MD) OSCC cases, staining intensity was weak in 60% of cases and strong in 40%. For poorly differentiated (PD) OSCC, 66.7% displayed weak staining intensity, with 33.3% showing strong staining. However, no notable relationship was observed between the various grades of OSCC and the staining intensity of reticulin fibers shown in Table 4.

**Table 4.** Correlation of Staining Intensity of Reticulin Fibres in Diverse Stage of OED and OSCC

| **Oral Epithelial Dysplasia** | | **Grade** | | | **Total** | **Spearman correlation** | |
|---|---|---|---|---|---|---|---|
| | | **Mild** | **Moderate** | **Severe** | | **r** | **p** |
| Staining intensity of reticulin fibres | Absent % | 15 | 15 | 13 | 43 | 0.246 | 0.08 |
| | | 100.0 | 100.0 | 86.7 | 95.6 | | |
| | Strong % | 0 | 0 | 2 | 2 | | |
| | | 0.0 | 0.0 | 13.3 | 4.4 | | |
| Total % | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |
| | | | | | | | |
| **OSCC** | | **Grade** | | | **Total** | **r** | **p** |
| | | **WD** | **MD** | **PD** | | | |
| Staining intensity of reticulin fibres | Weak% | 6 | 9 | 10 | 25 | -0.219 | 0.148 |
| | | 40.0 | 60.0 | 66.7 | 55.6 | | |
| | Strong % | 9 | 6 | 5 | 20 | | |
| | | 60.0 | 40.0 | 33.3 | 44.4 | | |
| Total % | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |

*r represents the Spearman correlation coefficient itself, which quantifies the strength and direction of the monotonic relationship between two variables.*
*p is the p-value associated with the Spearman correlation coefficient. This value assesses the statistical significance of the observed correlation.*

**Changes in collagen fibres;**

***Changes in collagen fibre birefringence***

Analysis of birefringence color of collagen fibers revealed distinct patterns across diverse stages of OED and OSCC. In mild OED cases, 53.3% exhibited a reddish color, while 40% had an orange color. Among moderate OED cases, 46.7% displayed an orange color, with 26.7% showing reddish and yellowish colors each. Severe OED cases showed varying colors, with 40% exhibiting yellowish-orange and green colors each, and 20% displaying reddish-orange color. For well-differentiated (WD) OSCC, 53.3% had an orange color, 20% had a reddish color, and 26.7% showed a yellowish color. Moderately differentiated (MD) OSCC cases predominantly displayed orange and yellowish colors (47.7% each), with a single case (6.7%) showing green color. Poorly differentiated (PD) OSCC cases predominantly exhibited green color (60%), with 26.7% showing yellowish color and 13.3% displaying orange color. A highly notable relationship ($p<0.001$) was observed between the various grades of OED and OSCC and the birefringence color of collagen fibers. Spearman correlation analysis indicated a moderate relationship among the various grades of lesions with the birefringence for collagen fibers (0.681 and 0.634, respectively) in OED and OSCC cases. This suggests that as the grade increases, the birefringence of collagen fibers tends to shift from reddish to green color depicted in Table 5.

 

**Table 5.** Correlation of Birefringence of Collagen Fibres in Diverse Stage of OED and OSCC

| **Oral Epithelial Dysplasia** | | **Grade** | | | **Total** | **Spearman correlation** | |
|---|---|---|---|---|---|---|---|
| | | **Mild** | **Moderate** | **Severe** | | **r** | **p** |
| Birefringence of collagen fibres | Red % | 8 | 4 | 0 | 12 | 0.681 | (<0.001)** |
| | | 53.3 | 26.7 | 0.0 | 26.7 | | |
| | Orange % | 6 | 7 | 3 | 16 | | |
| | | 40.0 | 46.7 | 20.0 | 35.6 | | |
| | Yellow % | 1 | 4 | 6 | 11 | | |
| | | 6.7 | 26.7 | 40.0 | 24.4 | | |
| | Green % | 0 | 0 | 6 | 6 | | |
| | | 0.0 | 0.0 | 40.0 | 13.3 | | |
| Total % | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |
| | | | | | | | |
| **OSCC** | | **Grade** | | | **Total** | **r** | **p** |
| | | **WD** | **MD** | **PD** | | | |
| Birefringence of collagen fibres | Reddish % | 3 | 0 | 0 | 3 | 0.634 | (<0.001)** |
| | | 20.0 | 0.0 | 0.0 | 6.7 | | |
| | Orange % | 8 | 7 | 2 | 17 | | |
| | | 53.3 | 46.7 | 13.3 | 37.8 | | |
| | Yellow % | 4 | 7 | 4 | 15 | | |
| | | 26.7 | 46.7 | 26.7 | 33.3 | | |
| | Green% | 0 | 1 | 9 | 10 | | |
| | | 0.0 | 6.7 | 60.0 | 22.2 | | |
| Total % | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |

*r represents the Spearman correlation coefficient itself, which quantifies the strength and direction of the monotonic relationship between two variables.*
*p is the p-value associated with the Spearman correlation coefficient. This value assesses the statistical significance of the observed correlation.*

## Changes in mucins;

### *Changes in the distribution pattern of acid mucins*

In the analysis of acid mucin distribution patterns, it was observed in Table 6 that mild Oral Epithelial Dysplasia (OED), 46.7% of cases exhibited a diffused distribution pattern, while 33.3% showed a focal distribution. Similarly, in moderate OED cases, 86.7% displayed a diffused distribution pattern, with 13.3% showing focal distribution. Severe OED cases predominantly showed a diffused distribution pattern (53.3%), with 26.7% exhibiting focal distribution. Among well-differentiated OSCC cases, 80% had a diffuse distribution pattern, and 13.3% showed focal distribution. Moderately differentiated (MD) OSCC cases also predominantly displayed a diffuse distribution pattern (80%), with 20% exhibiting focal distribution. Poorly differentiated (PD) OSCC cases showed similar patterns, with 46.7% displaying diffuse distribution and 40% showing focal distribution. However, no notable relationship was observed between the various grades of OED and OSCC and the distribution pattern of acid mucins.

**Table 6.** Correlation of Acid Mucins' Distribution Pattern in Diverse Stage of OED and OSCC

| **Oral Epithelial Dysplasia** | | **Grade** | | | **Total** | **Spearman correlation** | |
|---|---|---|---|---|---|---|---|
| | | **Mild** | **Moderate** | **Severe** | | **r** | **p** |
| Distribution pattern of acidic mucins | Absent % | 3 | 0 | 3 | 6 | 0.136 | 0.372 |
| | | 20.0% | 0.0% | 20.0% | 13.3% | | |
| | Diffuse | 7 | 13 | 4 | 24 | | |

 

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | % | 46.7% | 86.7% | 26.7% | 53.3% | | |
| | Focal % | 5 | 2 | 8 | 15 | | |
| | | 33.3% | 13.3% | 53.3% | 33.3% | | |
| Total % | | 15 | 15 | 15 | 45 | | |
| | | 100.0% | 100.0% | 100.0% | 100.0% | | |
| | | | | | | | |
| **OSCC** | | **Grade** | | | **Total** | **r** | **p** |
| | | **WD** | **MD** | **PD** | | | |
| Distribution pattern of acidic mucins | Absent % | 1 | 0 | 2 | 3 | 0.173 | 0.18 |
| | | 6.7 | 0.0 | 13.3 | 6.7 | | |
| | Diffuse % | 12 | 12 | 7 | 31 | | |
| | | 80.0 | 80.0 | 46.7 | 68.9 | | |
| | Focal (%) | 2 | 3 | 6 | 11 | | |
| | | 13.3 | 20.0 | 40.0 | 24.4 | | |
| Total (%) | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |

*r represents the Spearman correlation coefficient itself, which quantifies the strength and direction of the monotonic relationship between two variables.*
*p is the p-value associated with the Spearman correlation coefficient. This value assesses the statistical significance of the observed correlation.*

***Changes in the distribution pattern of neutral mucins***

In the assessment of neutral mucin distribution patterns, findings in Table 7 revealed that among mild Oral Epithelial Dysplasia (OED) cases, 53.3% exhibited a diffuse distribution pattern, while 26.7% showed focal distribution. In moderate OED cases, 80% displayed a diffuse distribution pattern, with 13.3% demonstrating focal distribution. Severe OED cases exhibited both diffuse and focal distribution patterns of neutral mucins in equal proportions (46.7% each). Among well-differentiated (WD) OSCC cases, 46.7% had a diffuse distribution pattern, and 40% showed focal distribution. Similarly, in moderately differentiated (MD) OSCC cases, 46.7% exhibited diffuse distribution, with 40% displaying focal distribution. In poorly differentiated OSCC cases, 60% displayed a diffuse distribution pattern, while 40% showed focal distribution. However, no notable relationship was observed between the various grades of OED and OSCC and the distribution pattern of neutral mucins.

**Table 7.** Correlation of Neutral Mucins' Distribution Pattern in Diverse Stage of OED and OSCC

| **Oral Epithelial Dysplasia** | | **Grade** | | | **Total** | **Spearman correlation** | |
|---|---|---|---|---|---|---|---|
| | | **Mild** | **Moderate** | **Severe** | | **r** | **p** |
| Distribution pattern of neutral mucins | Absent % | 3 | 1 | 1 | 5 | 0.221 | 0.144 |
| | | 20.0 | 6.7 | 6.7 | 11.1 | | |
| | Diffuse % | 8 | 12 | 7 | 27 | | |
| | | 53.3 | 80.0 | 46.7 | 60.0 | | |
| | Focal % | 4 | 2 | 7 | 13 | | |
| | | 26.7 | 13.3 | 46.7 | 28.9 | | |
| Total % | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |
| | | | | | | | |
| **OSCC** | | **Grade** | | | **Total** | **r** | **p** |
| | | **WD** | **MD** | **PD** | | | |
| Distribution pattern of neutral mucins | Absent % | 2 | 2 | 0 | 4 | 0.063 | 0.68 |
| | | 13.3 | 13.3 | 0.0 | 8.9 | | |

 

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Diffuse % | 7 | 7 | 9 | 23 | | |
| | | 46.7 | 46.7 | 60.0 | 51.1 | | |
| | Focal % | 6 | 6 | 6 | 18 | | |
| | | 40.0 | 40.0 | 40.0 | 40.0 | | |
| Total % | | 15 | 15 | 15 | 45 | | |
| | | 100.0 | 100.0 | 100.0 | 100.0 | | |

*r represents the Spearman correlation coefficient itself, which quantifies the strength and direction of the monotonic relationship between two variables.*
*p is the p-value associated with the Spearman correlation coefficient. This value assesses the statistical significance of the observed correlation.*

***Changes in staining intensity of acidic mucins***

In the examination of acid mucin staining intensity, it was found from Fig. 5 that among mild Oral Epithelial Dysplasia (OED) cases, 53.3% exhibited weak staining intensity, while 26.7% displayed strong staining intensity. In moderate OED cases, 80% showed strong staining intensity, with 20% demonstrating weak staining intensity. Severe OED cases predominantly displayed weak staining intensity (66.7%), with a minority (13.3%) exhibiting strong staining intensity. Among well-differentiated Oral Squamous Cell Carcinoma (OSCC) cases, 86.7% had strong staining intensity, while only 6.7% showed weak staining intensity. Moderately differentiated OSCC cases predominantly exhibited weak staining intensity (53.3%), with 26.7% showing strong staining intensity. For poorly differentiated OSCC cases, 66.7% displayed strong staining intensity, while 20% demonstrated weak staining intensity. However, no notable relationship was observed between the various grades of OED and OSCC and the staining intensity of acid mucins.

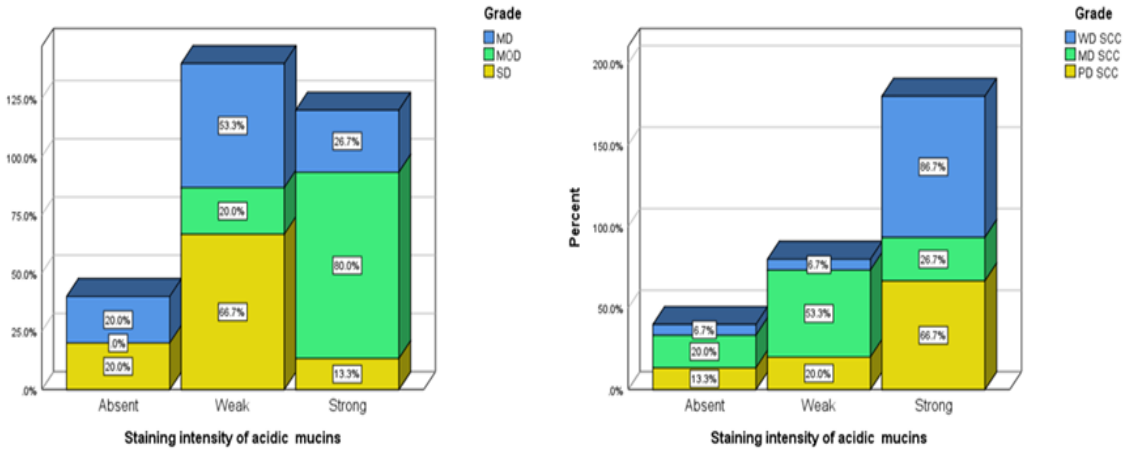


**Fig. 5:** *Correlation of Acidic Mucins' Staining Intensity in Diverse Stage of OED and OSCC*

***Changes in staining intensity of neutral mucins***

In the assessment of neutral mucin staining intensity, it was observed in Fig. 6 that among mild Oral Epithelial Dysplasia (OED) cases, 53.3% exhibited weak staining intensity, while 26.7% displayed strong staining intensity. Similarly, in moderate OED cases, 86.7% showed weak staining intensity, with only 6.7% demonstrating strong staining intensity. Severe OED cases predominantly displayed weak staining intensity (80%), with 13.3% exhibiting strong staining intensity. Among well-differentiated Oral Squamous Cell Carcinoma (OSCC) cases, 46.7% had strong staining intensity, while 40% showed weak staining intensity. Moderately differentiated OSCC cases predominantly exhibited strong staining intensity (40%), with 46.7% showing weak staining intensity. For poorly differentiated OSCC cases, 73.3% displayed strong staining intensity, while 26.7% demonstrated weak staining intensity. However, no notable relationship was observed between the various stages of OED and OSCC and the staining intensity of neutral mucins.

 

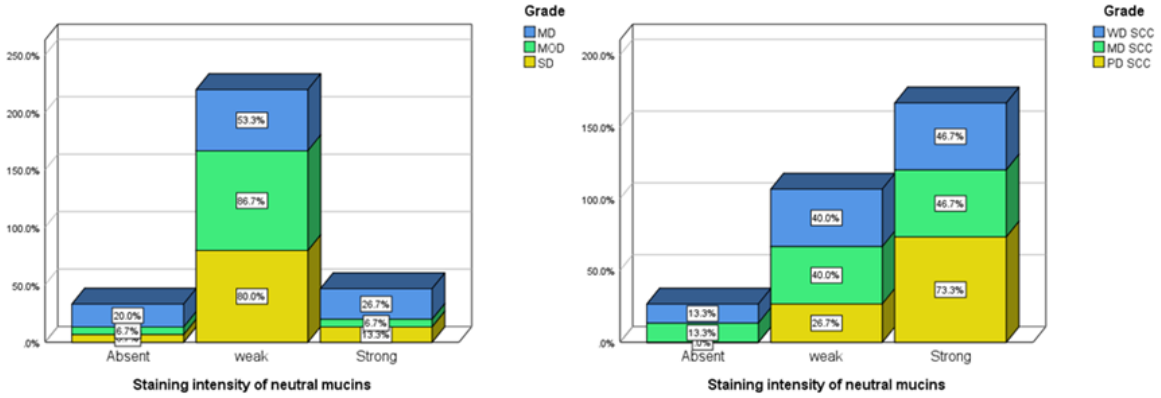


**Figure 6.** Correlation of Neutral Mucins' Staining Intensity in Diverse Stage of OED and OSCC

## CONCLUSION

The conclusion drawn from the current research underscores the pivotal role of stroma in the genesis and progression of two significant oral pathologies: oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC). Through meticulous examination and analysis, it becomes evident that stromal alterations contribute substantially to the development and advancement of these conditions. This observation holds profound implications for both diagnosis and treatment strategies in oral oncology.One notable aspect highlighted by the findings is the utility of special stains in elucidating stromal involvement. These stains offer a cost-effective alternative to more sophisticated molecular techniques, rendering them accessible for widespread adoption in various laboratory settings. Moreover, their reliability in providing diagnostic insights underscores their significance in clinical practice. By leveraging these stains, clinicians can gain valuable insights into the polymeric characteristics and advancements underlying stromal contributions to OED and OSCC. This not only enhances our understanding of the diseases but also facilitates more informed decision-making regarding patient management. Furthermore, the study emphasizes the importance of further research endeavors aimed at unraveling the molecular mechanisms and nanotechnological biomarkers associated with stromal alterations in oral carcinogenesis. Such investigations hold the promise of uncovering novel therapeutic targets and diagnostic biomarkers, thus paving the way for more effective treatment modalities and precision medicine approaches. By delving deeper into the intricate interplay between stroma and epithelial cells, researchers can uncover crucial insights that may ultimately translate into tangible clinical benefits for patients. In addition to advancing our understanding of the underlying pathophysiology, prospective studies are warranted to validate the diagnostic and prognostic utility of histochemical polymeric investigation in clinical settings. These studies hold the potential to bridge the gap between research findings and real-world applications, thereby facilitating the integration of novel diagnostic modalities into routine clinical practice. By rigorously evaluating the efficacy and reliability of these investigative techniques in diverse patient populations, clinicians can confidently incorporate them into their diagnostic algorithms, thus improving patient outcomes and enhancing overall healthcare delivery in the field of oral oncology.

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