BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 5

DOI: 10.58240/1829006X-2025.21.5-66

ORIGINAL RESEARCH

ASSESSING TUMOR MICROENVIRONMENT OF ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA IN ORAL SUBMUCOUS FIBROSIS USING MASSON'S TRICHROME.

Vimalasubhashini Vivekbalamithran[1], Abilasha Ramasubramanian[1*]

[1.] Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India.

***Corresponding Author:** Abilasha Ramasubramanian, Professor, Department of Oral Pathology, Saveetha Dental College and Hospital, Chennai, TamilNadu, India. E-mail: abilasha@saveetha.com

Received: *May 5, 2025;* ***Accepted:*** *June. 6 , 2025;* ***Published:*** *June. 15,2025*

ABSTRACT

Background: Oral submucous fibrosis (OSMF) is a chronic, progressive disease that affects both epithelial and connective tissue structures. Grading according to various histological parameters which include the epithelial and the connective tissue features can be demonstrated in routine hematoxylin and eosin stains(H&E). However, the use of special stains ensures better visualization which aids in early diagnosis of these connective tissue changes which includes microenvironmental changes and epithelial mesenchymal transition especially in advanced OSMF cases. The present study aims to compare the micro environmental changes and epithelial mesenchymal transition by evaluation of muscle involvement and degeneration in cases with advanced oral submucous fibrosis using Masson's trichrome

Methods: Grouping: Group 1: OED with OSMF (5 cases) Group 2: OSCC with OSMF (5 cases) Total 10 cases were taken and stained with both H&E staining and Masson's trichrome stain. This helps in assessing tumor microenvironment of oral epithelial dysplasia and oral squamous cell carcinoma in Oral submucous fibrosis using Masson's trichrome.

Results: This study shows higher amounts of inflammation in OSMF with the OSCC group, as it can be easily differentiated and helps in identifying malignant transformation. Among the two groups, oral epithelial dysplasia with Oral submucous fibrosis shows more fibrosis extension into the muscle.

Conclusion: The questionable areas of degenerating muscle bundles or areas of hyalinization were better distinguished with Masson's stain, especially in deeper connective tissue and was statistically significant. The purpose of this study is to identify potentially malignant diseases from malignant transformation of dysplasia and helps to study how collagen deposition can affect oral squamous cell carcinoma.

Keywords: Masson's trichrome, Oral submucous fibrosis, Oral epithelial dysplasia, Oral squamous cell carcinoma.

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, progressive disease that affects both epithelial and connective tissue structures, and is brought on by having Areca nuts in betel quid or its derivatives [1]. Chewing smokeless tobacco, eating foods high in copper, vitamin deficiencies[2], malnutrition resulting in low blood protein levels, anemia, and genetic predisposition are all considered contributing risk factors[3,4].

Different histological criteria, such as alterations in connective tissue and epithelium, are used to grade oral submucous fibrosis (OSMF). Epithelial changes like epithelial Atrophy, Epithelium thinning is frequently observed and may result in increased susceptibility to trauma[5]. Hyperplasia and Hyperkeratosis, In certain instances, there may be both hyperkeratosis (thinning of the outer keratin layer) and hyperplasia (thickening of the epithelium)[6].

Dysplasia, the existence of precancerous modifications in the cells known as dysplastic changes[7], which suggest the possibility of malignant transformation[6,8]. Then subepithelial connective tissue changes, the process of hyalinization, in which the tissue becomes glassy and homogenous as a result of excessive collagen deposition, is frequently seen in the subepithelial connective tissue. The submucosal tissues exhibit extensive fibrosis due to a substantial increase in collagen deposition. The oral tissues have become less flexible and more rigid due to an overabundance of collagen. Thick, fibrous band formation in the submucosal tissues: This condition is linked to trismus, or limited mouth opening[9,10].

In the subepithelial layer, early stages frequently exhibit a persistent inflammatory cell infiltration, primarily composed of lymphocytes and plasma cells[8]. As the fibrosis gets larger in mature stages, the inflammatory response usually decreases. Within fibrotic areas, there is a decrease in the quantity of blood vessels. Pressure from the surrounding dense collagen fibers might make the existing capillaries appear restricted or destroyed. These histological characteristics are essential for the diagnosis and treatment of OSMF because they help distinguish the disorder from other fibrotic and precancerous conditions of the oral cavity and provide light on the disease's extent[11]. Monitoring OSMF progression and probable malignant transformation requires routine biopsy and histological investigation[12].

Regular Hematoxylin and Eosin (H and E) staining could be used to identify these characteristics[13]. But in advanced OSMF cases, the application of certain stains helps to better visualize and early diagnosis of connective tissue changes like transforming fibroblasts into myofibroblasts and epithelial mesenchymal transition by providing a contrast to different components of the connective tissue[11]. We are projecting connective tissue components in this study using Masson's trichrome. Histology uses Masson's Trichrome stain, a three-color staining technique, to identify muscle, collagen, and fibrin in tissue sections[14]. For the histological assessment of disorders such as oral submucous fibrosis (OSF), it is particularly useful in analyzing fibrotic alterations[15]. The distinct hue differentiation makes it possible to see various tissue components clearly, Black-blue nuclei,cytoplasm, keratin, and muscle fibers in red color, mucin and collagen fibers in blue color[16].

Collagen fibers blue staining makes it simple to determine the degree of fibrosis [17] and to identify it, aids in comprehending the structural alterations in the mucosa and in differentiating between various tissue types, through contrasting the level of tissue fibrosis and collagen deposition across time, or before and after treatment[18]. This study compares and uses Masson's trichrome to assess muscle degeneration and participation in advanced oral submucous fibrosis.

To compare and evaluate the microenvironment of fiber and collagen deposition in Oral submucous fibrosis, sections were taken from the department block archives of patients already diagnosed with different grades of oral submucous fibrosis, patients(n=10) were grouped into two groups , group 1(n=5) Oral epithelial dysplasia with Oral submucous fibrosis(figure 1) and group 2(n=5) Oral squamous cell carcinoma with Oral submucous fibrosis (figure 2). All the ten cases were stained with both hematoxylin and eosin(H&E) staining and Masson's trichrome stain (figure 3). Staining is done with a Masson's trichrome kit. After staining all the slides were dried and are mounted with a dibutyl phthalate polystyrene xylene medium. In all the slides few parameters have been evaluated; they are fibrosis extending into muscle, vascularity, inflammation and pattern of collagen.

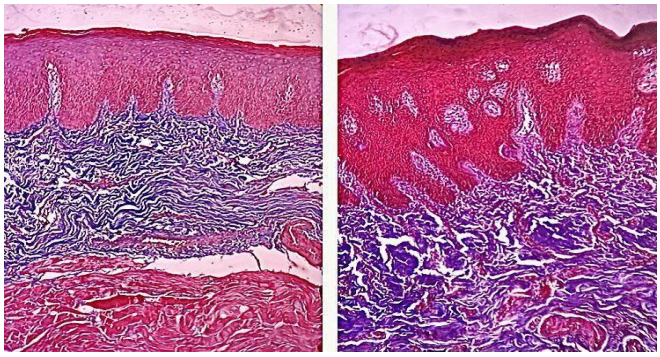


Figure 1. Masson trichrome stained sections of group 1(i.e; oral epithelial dysplasia with oral submucous fibrosis).

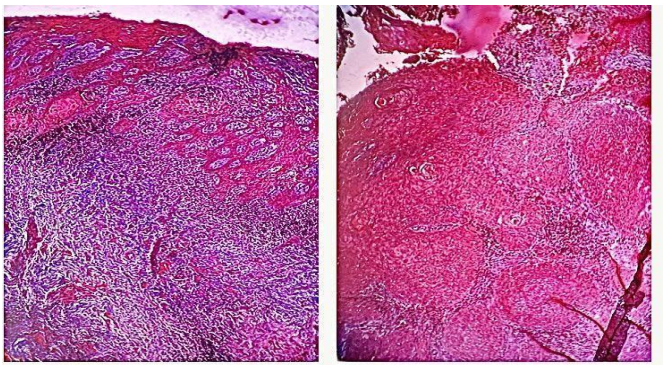


Figure 2. Masson trichrome stained sections of group 2 (i.e; oral squamous cell carcinoma with oral submucous fibrosis).

RESULT

Various parameters were evaluated and recorded like fibrosis extending into muscle, vascularity, inflammation,

pattern of collagen. Among different sets of cases, tabulation has been interpreted (table 1 and table 2). Fibrosis extension into muscle shows increased in group 2(+present, -absent). Vascularity; Shows more or less similar in both the groups (+mild,++moderate,+++intense).

Inflammation; Higher in group 2 when compared to group 1(+mild, ++moderate, +++intense). Pattern of collagen; predominantly hyalinization in group 1 while wavy in group 2(1-wavy, 2-hyalinized). Dense, widespread fibrosis with nearly total hyalinization and little inflammation were evident in the advanced stages of oral submucous fibrosis.

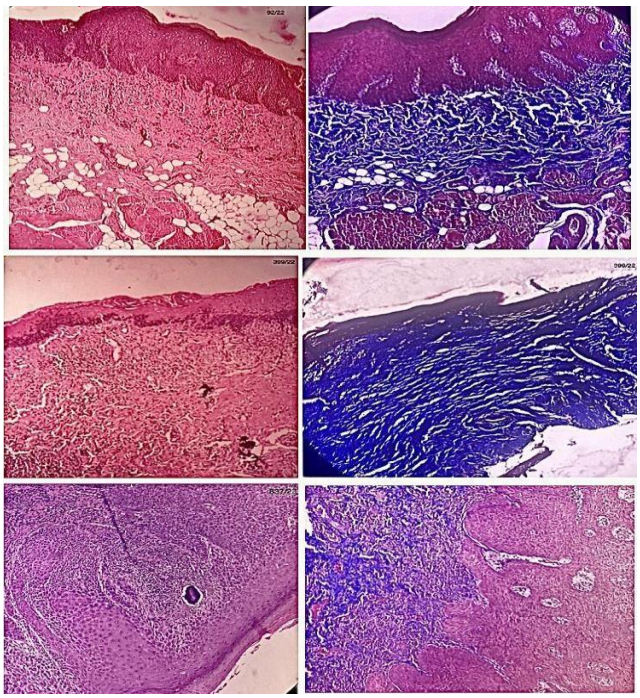


Figure 3. Both the groups stained with hematoxylin and eosin stain(on the left side) and masson's trichrome staining (on the right side)

Table 1. Table shows varying levels of collagen and fibrosis in five different patients with oral epithelial dysplasia with oral submucous fibrosis.

OED with OSMF (Group 1)	Fibrosis extending into muscle	Vascularity	Inflammation	Pattern of collagen
1	-	++	++	1
2	+	++	+	2
3	+	++	+	1
4	+	++	+	2
5	+	+	+	2

Table 2. Table shows varying levels of collagen and fibrosis in five different patients with oral squamous cell carcinoma with oral submucous fibrosis.

OSCC with OSMF (Group 2)	Fibrosis extending into muscle	Vascularity	Inflammation	Pattern of collagen
1	-	++	++	2
2	+	++	+++	2
3	-	++	+++	1
4	-	++	+++	1
5	+	++	++	2

TABLE 3. REPRESENTS THE DISTRIBUTION OF STUDY PARAMETERS IN THE STUDY GROUPS

STUDY PARAMETERS	SCORES	STUDY GROUPS	
		GROUP 1	GROUP 2
Fibrosis into muscle	0	1	3
	1	4	2
Vascularity	1	1	0
	2	4	5
Inflammation	1	4	0
	2	1	2
	3	0	3
Pattern of collagen	1	2	2
	2	3	3

Table 3 and Graph 1 presents the distribution of study parameters across two study groups. In terms of fibrosis into muscle, Group 1 had more cases with a score of 1 (4 cases) compared to Group 2 (2 cases), while Group 2 had a higher number of cases with a score of 0 (3 cases). For vascularity, most participants in both groups had a score of 2, with Group 1 having 4 cases and Group 2 having 5 cases. Regarding inflammation, Group 1 had more cases with lower scores (4 cases with score 1 and 1 case with score 2), while Group 2 had a more even distribution across scores 2 (2 cases) and 3 (3 cases). The pattern of collagen was similarly distributed across both groups, with slight variations. Overall, the distribution of parameters indicates differences in fibrosis, vascularity, and inflammation between the two groups, potentially suggesting variations in tissue characteristics or responses.

GRAPH 1 DISTRIBUTION OF STUDY PARAMETERS IN THE STUDY GROUPS

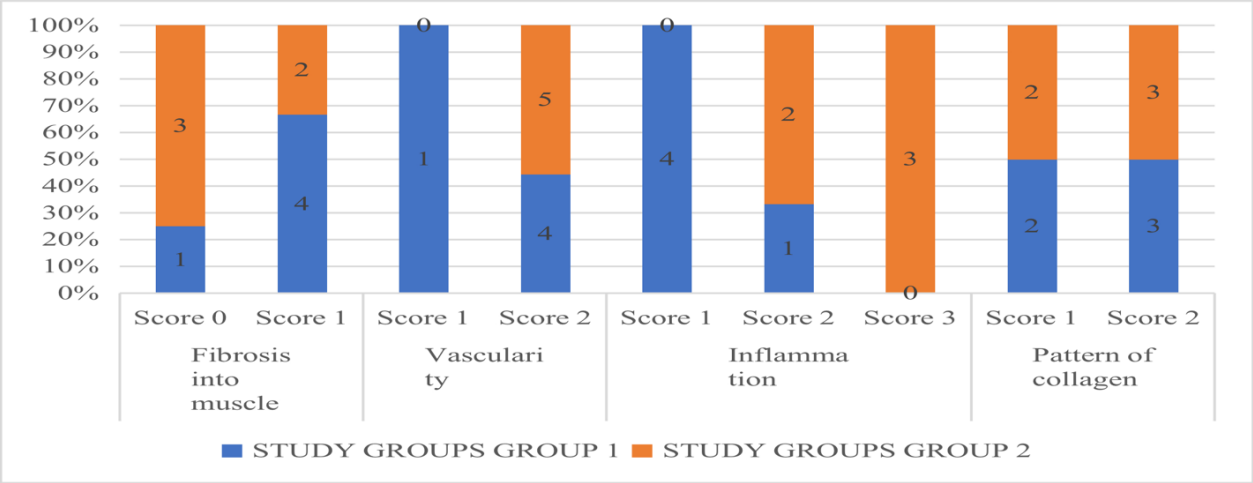


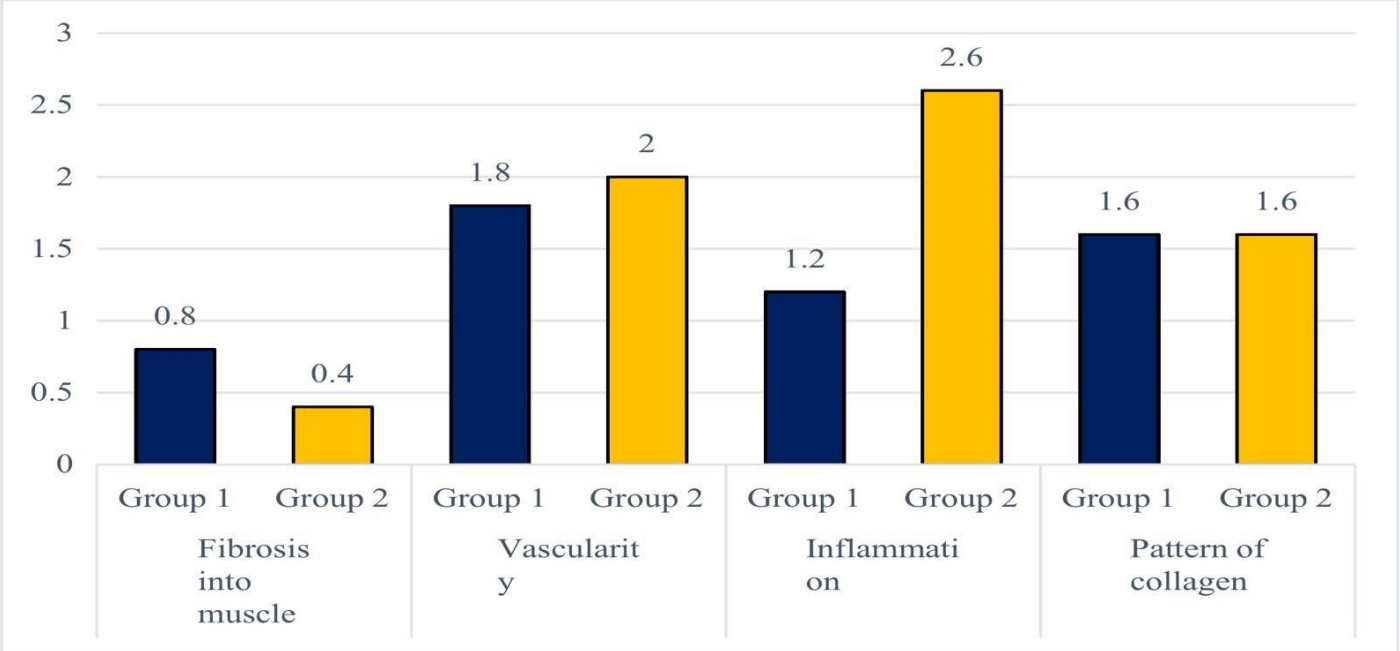
TABLE 4. REPRESENTS THE INTERGROUP COMPARISON BETWEEN STUDY GROUPS AND STUDY PARAMETERS

STUDY PARAMETERS	GROUPS	MEAN	S.D	SIG	95% CONFIDENCE INTERVAL	
					LOWER	UPPER
Fibrosis into muscle	**1**	.80	.447	.242	-.329	1.129
	2	.40	.548			
Vascularity	**1**	1.80	.447	.347	-.661	.261
	2	2.00	.000			
Inflammation	**1**	1.20	.447	.002*	-2.129	-.671
	2	2.60	.548			
Pattern of collagen	**1**	1.60	.548	1.000	-.799	.799
	2	1.60	.548			

***P value less than or equal to 0.05 is considered statistically significant different**

Table 4 and Graph 2 presents the intergroup comparison of study parameters between the two study groups. The results show no statistically significant differences in fibrosis into muscle (p = .242), vascularity (p = .347), or pattern of collagen (p = 1.000) between the groups. However, inflammation showed a statistically significant difference (p = .002), with Group 2 having a higher mean score (2.60 ± 0.548) compared to Group 1 (1.20 ± 0.447), indicating greater inflammatory response in Group 2. The confidence intervals further support these findings, particularly highlighting the significant difference in inflammation levels between the groups. Overall, while most parameters did not show significant variation, the increased inflammation in Group 2 suggests a noteworthy difference in the biological response between the groups.

GRAPH 2 REPRESENTS THE INTERGROUP MEAN COMPARISON BETWEEN STUDY GROUPS AND STUDY PARAMETERS



DISCUSSION

The chronic and severe condition known as oral submucous fibrosis (OSMF) is typified by the gradual fibrosis and inflammation of the submucosal tissues, which include the lamina propria and deeper connective tissues. It leads to noticeable stiffness and eventually the incapacity to open the mouth. The disorder is especially linked to chewing Areca nuts and is well known for its carcinogenic potential. Since the pathophysiology of OSMF is poorly understood, it is thought to be complex[1].

Possible mechanism of OSMF transforming into malignancy is illustrated by kumar bijay et al stated that Areca nut chewing will cause initiation of osmf which can be identified clinically by burning sensation (histamine) and excessive salivation (chymase) where mast cell density will increase and then release of histamine and chymase followed by release of fibroblast growth factor, vascular endothelial growth factor and transitional growth factor-ß leads to fibrosis and trismus clinically, finally malignant transformation occurs by mast cell tryptase and chymase, proliferation of myofibroblast, angiogenesis and gene mutation[19].

Another study done by kizhakkoottu et al, also stated the histopathogenesis of malignant transformation of OSMF[20]. The collagen fibers that display perivascular cuffing around blood arteries may have extravasated inflammatory cells surrounding them[8]. The surrounding collagen fiber's tendency to be hyalinized causes the blood arteries to constrict. Decreased levels of angiogenesis mediators lead to a decrease in micro vessel density as fibrosis and hyalinization advance. Clinically, these alterations manifest as mucosal blanching. Masson's stain provided a statistically significant distinction between the areas of hyalinization or degenerating muscle bundles that were dubious, particularly in deeper connective tissue. This study aims to distinguish between potentially malignant disorders and the carcinogenic transition of dysplasia and advances research on the potential impact of collagen deposition on oral squamous cell cancer[21]. Masson's Trichrome stains collagen in blue; muscle, cytoplasm and erythrocytes in red; and nuclei in blue/black color stain. This characteristic in OSMF is notably improved, making it simple to identify the dense and excessive collagen fibers that define the fibrotic bands.

Determining the degree of fibrosis can be made easier by seeing the extent of collagen deposition. The uniformity and density of the collagen are shown by the blue staining of the hyalinized areas of the subepithelial connective tissue. This characteristic is especially helpful in identifying regions with extensive fibrosis. Angiogenesis as discussed earlier plays a crucial role in tumor microenvironment which can be elevated in red color. Few inflammatory cells like mast cells, tumor associated fibroblasts can also be differentiated.

This aids in the evaluation of the tumor microenvironment in oral Submucous fibrosis for oral squamous cell carcinoma and oral epithelial dysplasia. The OSMF group in this study had higher levels of inflammation than the OSCC group, which is immediately distinguishable and aids in the detection of malignant transformation. There is greater fibrosis extension into the muscle in oral epithelial dysplasia with oral submucous fibrosis group of patients.

Pathologists find Masson's Trichrome stain to be an indispensable tool in the diagnosis of OSMF since it allows for the vivid and unmistakable distinction between different tissue types. The improved ability to see fibrotic alterations and tissue architecture aids in staining the stage of the disease by emphasizing the degree and density of fibrosis. Understanding the evolution of OSMF or the response to treatment can be aided by comparing stained sections across time. Differentiating OSMF from other oral mucosal diseases and ailments that have similar clinical presentations but different histological findings is made easier by the staining pattern's distinctive appearance.

CONCLUSION

Masson's trichrome stain was statistically significant and allowed for a better distinction between the areas of hyalinization or degenerating muscle bundles, particularly in deeper connective tissue, angiogenesis and tumor associated fibroblast and macrophages. This helps to identify epithelial mesenchymal transition. Future developments in staining techniques will even allow for the differentiation of various inflammatory cells. Differentiating cells using different stains would be recommended to facilitate further work. Aspects that affect virtual assistant deployment in dental practice. Research needs to conduct comprehensive qualitative examinations for revealing the detailed workplace attitudes of dental staff regarding virtual assistant technology adoption in their clinical work environment.

CONCLUSION

Patients together with workflow efficiency experienced significant improvement in dental practice through AI-driven virtual assistants. Thorough examination of quantitative alongside qualitative information in the study showed major enhancements affecting domains from patient satisfaction results to clinical results as well as appointment bookings and administrative performance time alongside operational workflow efficiency. The research demonstrates how AI technology will transform dental practice management to give clinicians enhanced power for delivering quality healthcare with optimized operational performance. The identification of patient satisfaction score and staff attitude predictors offers important directions for deploying strategies that yield optimal support for continuous improvement.

DECLARATIONS

Funding
The work was not funded.

Ethical Approval
"Not applicable"

Consent for publication
"Not applicable"

Ethical approval
None

Competing interest
The authors declare that there are no competing interest.

Acknowledgements
None

REFERENCES

1. Ranjitham PS, Kuzhali S, Sumathy MP, Gnandeepam S: Evaluation of Platelet Parameters in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma in a Background of Oral Submucous Fibrosis. Indian J Dent Res. 2023, 34:417–21.

2. Sagar S, Raman P, Gheena S, Abilasha R, Krishnan RP, Selvaraj J: Salivary vitamin D levels among OSCC and normal Indian patients. Bioinformation. 2022, 18:884–7.

3. Liu Z, Hu W, Shan Z, Liu S, Yao Z, Quan H: Evaluation of stromal myofibroblasts in oral submucous fibrosis and its malignant transformation: An immunohistochemical study. J Cancer Res Ther. 2024, 20:706–11.

4. Kotecha A: Oral submucous fibrosis: a guide to diagnosis and management. Br Dent J. 2024, 236:598.

5. Wang SY, Zhang SJ, Meng HF, et al.: DPSCs regulate epithelial-T cell interactions in oral submucous fibrosis. Stem Cell Res Ther. 2024, 15:113.

6. Chen X, Chen T, Xie H, Guo J: Salivary and serum levels of lactate dehydrogenase in oral submucous fibrosis: A meta-analysis. Medicine . 2024, 103:e37788.

7. Vijayashree Priyadharsini J, Abilasha R, Anitha P, Paramasivam A: Engineered Dex: A promising therapeutic strategy for oral squamous cell carcinoma. J Stomatol Oral Maxillofac Surg. 2023, 124:101277.

8. Srivastava R, Singh DK, Thomas PA, Raza H, Sangamesh NC, Bagde HS: Comparative Analysis of Tissue Copper Levels in Oral Submucous Fibrosis (OSMF) Patients. J Pharm Bioallied Sci. 2024, 16:S629–31.

9. Rai A, Parveen S, Shree P, et al.: Salivary transforming growth factor beta in oral submucous fibrosis: A diagnostic and predictive marker. J Cancer Res Ther. 2024, 20:275–80.

10. Warnakulasuriya S, Ranganathan K: Oral Submucous Fibrosis: A Guide to Diagnosis and Management. Springer Nature; 2023.

11. Kizhakkoottu S, Ramani P, Tilakaratne WM: Mesenchymal stem cells in the management of oral submucous fibrosis-An enigma to be solved. J Oral Pathol Med. 2024, 53:275–6.

12. Dongarwar G, Bhowate R, Degwekar S: Oral Submucous Fibrosis: An Update. LAP Lambert Academic Publishing; 2013.

13. Esfehani R, Khadivi F, Valipour J, et al.: Secretome of human amniotic membrane stem cells promote recovery and testicular functions through modulating pathway in mice testicular torsion: An experimental study. Int J Reprod Biomed. 2024, 22:821–36.

14. Bauman TM, Nicholson TM, Abler LL, Eliceiri KW, Huang W, Vezina CM, Ricke WA: Characterization of fibrillar collagens and extracellular matrix of glandular benign prostatic hyperplasia nodules. PLoS One. 2014, 9:e109102.

15. Aljohani K, Alqarni A, Harte M, Alghamdi R, Alzahrani S, Albuquerque R: Oral Potentially Malignant Disorders and Oral Cancer in Saudi Arabia: An Epidemiological Review of the Literature. J Clin Med Res. 2024, 13.: 10.3390/jcm13051376

16. Gao L, Zhuang Y, Liu Z: Reducing Nogo-B Improves Hepatic Fibrosis by Inhibiting BACe1-Mediated Autophagy. Tissue Eng Regen Med. Published Online First: 17 April 2024. 10.1007/s13770-024-00641-5

17. Morris RA, Dubielzig RR: Light-microscopy evaluation of zonular fiber morphology in dogs with glaucoma: secondary to lens displacement. Vet Ophthalmol. 2005, 8:81–4.

18. Yan L, Liang B, Feng J, Zhang H-Y, Chang H-S, Liu B, Chen Y-L: Safety and feasibility of irreversible electroporation for the pancreatic head in a porcine model. World J Gastrointest Oncol. 2022, 14:1499–509.

19. Website. Accessed: Bijai, L.K., Aboalela, A.A., Albalawi, F. et al. Role of mast cells in the pathogenesis of oral submucous fibrosis: a systematic review and meta-analysis. BMC Oral Health 24, 1219 (2024). https://doi.org/10.1186/s12903-024-05025-8.

20. Kizhakkoottu S, Ramani P, Tilakaratne WM: Role of Stem Cells in the Pathogenesis and Malignant Transformation of Oral Submucous Fibrosis. Stem Cell Rev Rep. 2024, 20:1512–20.

21. Reichal P, Ramani P, Kizhakkoottu S: Association of Site and Recurrence in Oral Squamous Cell Carcinoma Patients Visiting Private Hospital in Chennai: A Retrospective Study. Cureus. 2024, 16:e52774.