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Enhanced ZEB 1 stromal expression is a marker for epithelial mesenchymal transition in oral submucous fibrosis

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ABSTRACT

Introduction: Oral submucous fibrosis is a progressive oral mucosal condition that is characterized by inflammation and persistent fibrosis. Epithelial Mesenchymal Transition is a crucial molecular event that contributes to tumor progression and fibrosis, with ZEB 1 and its effect on E-cadherin expression being key molecules in the process. Thera are no tissue level studies of these molecules in oral submucous fibrosis.
Objective: To evaluate the immunohistochemical expression of epithelial mesenchymal transition markers E-cadherin and ZEB1 in oral submucous fibrosis.
Methodology: A total of 30 cases of Oral submucous fibrosis (15 Early OSMF and 15 Advanced OSMF) classified based on the histopathological features were included in the study. Immunohistochemistry was done using two markers i.e. E-cadherin and ZEB1. The difference in the expression of E-Cadherin and ZEB1 among histopathological grades of OSMF was done by Mann-Whitney U test.
Results: A slight reduction in the E-cadherin expression was noted in Oral submucous fibrosis but marked enhanced expression of ZEB1 was seen in the connective tissue of OSMF.
Conclusion: An increase in intensity and percentage of positivity of ZEB 1 expression in connective tissue was observed in advanced cases as compared to early OSMF. This can be attributed to role of ZEB1 in mediating EMT via transdifferentiation of fibroblast into myofibroblast and thus predispose to fibrosis in OSMF.

1. Introduction

Oral submucous fibrosis (OSMF) is a progressive oral mucosal disease characterized by inflammation and persistent fibrosis of the submucosal compartment.[1] Oral submucous fibrosis causes significant rigidity leading to difficulty in opening the mouth. It most commonly affects the buccal mucosa, but can involve the other parts of oral cavity and also occasionally extend the pharynx.[1,2] It has been recently defined by More C and Rao N, encompassing both the clinical and histopathological features as 'a debilitating, progressive, irreversible collagen metabolic disorder induced by chronic chewing of areca nut and its commercial preparations; affecting the oral mucosa and occasionally the pharynx and oesophagus; leading to mucosal stiffness and functional morbidity; and has a potential risk of malignant transformation".[3] The prevalence of OSMF ranges from 0.1 to 30 % as per a recent review and has been associated with smokeless tobacco and arecanut in its various forms.[4,5]

It is widely recognized as a "potentially malignant disorder" as patients have been reported to have increased risk of developing oral malignancy.[6] Apart from that, OSMF may be associated with several systemic diseases involving multiple systems adding to the morbidity associated with the lesion.[5]

Histopathologically, it is characterized by varying degree of fibrosis and hyalinization of collagen fibers of underlying connective tissue stroma with atrophy of surface epithelium.[5] The pathogenesis of OSMF is not clearly understood, but there is compelling evidence to suggest that OSMF is a result of collagen deregulation.[2,7] Therefore, an increase in collagen formation concomitant with reduced collagen degradation is one of the plausible explanations for the onset of this condition.[5,7] EMT is known to play a major role in organ fibrosis and also been implicated in OSMF. It has been described that the pathological changes in the connective tissue of OSMF are likely to affect the overlying epithelium and induce EMT.[6,7] The inflammatory reaction antecedent to fibrosis and the role of EMT in fibrogenesis and malignant transformation in

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other organs, points to the involvement of EMT in the pathogenesis of OSMF and its malignant transformation.[7] The inflammatory cytokines produced in response to the inflammation may mediate the progression of OSMF via various EMT pathways. The membranous loss of E-cadherin, beta-catenin, Cytokeratin 5, and Cytokeratin 14 with an overwhelming expression of vimentin, *N*-cadherin and alpha- Smooth muscle actin seen in OSMF further confirms the role of EMT in OSMF.[7–12] ZEB1, a transcription factor has been associated with EMT through regulation of target genes via its protein binding domains, especially that of E-Cadherin.[13–15] ZEB1, thus has a vital role in the down-regulation of E-cadherin, a key event predisposing to EMT.[16]

An alarming complication associated with OSMF is the higher risk of transforming to oral squamous cell carcinoma.[9,17–23] Identification of signature genes influencing EMT may unravel novel pathways, which are critical for development of fibrosis in OSMF and its progression to oral cancer.[24–26] A thorough understanding of signaling pathways involved in EMT and the tumor microenvironment in OSMF and OSCC can pave way for newer strategies for management. Till date, there are only limited studies on clinical significance or co-relation between ZEB1 and E-Cadherin in OSCC, while no studies exist on potentially malignant disorders of oral cavity especially oral submucous fibrosis.

With this background, the present study aimed to evaluate the expression of two well-known EMT markers E-Cadherin and ZEB1 in OSMF.

2. Methodology

Following institutional ethical approval, the retrospective observational study was done using 30 paraffin embedded tissue blocks of clinically and histologically proven cases of Oral Submucous Fibrosis. The cases used in the study were retrieved from the Archives of Department. The study was conducted between December 2021 to December 2022. Three tissue sections of 4 μm each were cut from each block and taken onto "amino propyl triethoxysilane (APES)" coated slides. One slide was stained with Hematoxylin and eosin. While the other slides were stained Immunohistochemically using antibody against ZEB1 (1:100 ThermoFischer Scientific USA) and E-Cadherin (Pre-diluted PathnSitu) followed by detection Using PolyExcel HRP/DAB Detection System Two Step Universal Kit (PathnSitu Catalogue no #PEH002/USA). The control slides were run with all sets of immunostaining which were Normal epithelium for E− Cadherin and Intralesional Lymphocytes for ZEB-1. The clinical data of the cases was collected and tabulated from the archival registers (Table 1). In all the cases, the site of biopsy was from the buccal mucosa. The oral submucous fibrosis tissue sections were subdivided histopathologically into very early, early, moderately advanced and advanced, using criteria of Pindborg and Sirsat[27] which is based on the type of collagen, number of fibroblasts, blood vessel morphology and the number of inflammatory cells. The very early and early OSMF were categorized as "Early OSMF" (15 cases) and moderately advanced and advanced OSMF into the category of "Advanced OSMF" (15 cases) (Fig. 1). After the slides were immuno-stained, they were evaluated by two oral pathologists and their observations were tabulated. Any disparity was assessed again in the penta-headed multi-viewing microscope to reach a common consensus. The slides were analysed on three main criteria; intensity, localization and percentage of positivity staining. The statistical analysis was done using SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY) and values of $P < 0.05$ were considered statistically significant. The difference in the expression of E–Cadherin and ZEB-1 in epithelium and Connective tissue in histopathological grades of OSMF (early & advanced OSMF) with regards to intensity, location and percentage of positivity was evaluated using Mann Whitney Test.

Table 1
Clinical and Histopathological features of the Oral submucous fibrosis cases.

Sl. No	Age	Gender	Habit	Histopathological diagnosis
1	33	M	Areca nut chewing	Early OSMF
2	41	M	Gutkha Chewing	Early OSMF
3	48	M	Gutkha Chewing	Early OSMF
4	38	M	Gutkha Chewing	Early OSMF
5	32	M	Gutkha Chewing	Early OSMF
6	38	M	Tobacco Chewing	Early OSMF
7	35	M	Chewing Tobacco	Early OSMF
8	20	M	Gutkha Chewing	Early OSMF
9	40	M	Gutkha Chewing	Early OSMF
10	33	M	Areca nut chewing	Early OSMF
11	60	M	Areca nut chewing	Early OSMF
12	32	M	Gutkha Chewing	Early OSMF
13	37	M	Gutkha Chewing	Early OSMF
14	25	M	Gutkha Chewing	Early OSMF
15	18	M	Gutkha Chewing	Early OSMF
16	22	M	Gutka chewing	Advanced OSMF
17	24	M	Gutka chewing	Advanced OSMF
18	42	M	Gutka chewing	Advanced OSMF
19	21	M	Gutkha Chewing	Advanced OSMF
20	24	M	Betel leaf and Areca nut consumption	Advanced OSMF
21	19	M	Areca nut chewing	Advanced OSMF
22	23	M	Gutkha Chewing	Advanced OSMF
23	35	M	Gutkha Chewing	Advanced OSMF
24	52	M	Chewing Tobacco	Advanced OSMF
25	38	M	Areca nut chewing	Advanced OSMF
26	45	M	Gutkha Chewing	Advanced OSMF
27	30	M	Gutkha Chewing	Advanced OSMF
28	44	M	Gutkha Chewing	Advanced OSMF
29	38	M	Gutka chewing	Advanced OSMF
30	32	M	Gutka chewing	Advanced OSMF

3. Results

All the cases of OSMF showed positivity for E cadherin and ZEB1 markers with varying intensity, percentage of positivity and location of staining.

3.1. Comparison of E-cadherin expression in histopathological grades of OSMF by Mann-Whitney U test (Table 2) (Fig. 2)

Intensity – In early OSMF, 5 (33 %) cases showed mild & 10 (77 %) cases showed intense staining. In advanced OSMF, all 15 (100 %) cases showed intense staining. A progressive increase in intensity from early to advance OSMF was observed but did not reach statistical significance (p-value was 0.1249).

Location – All cases (100 %) of OSMF in both subgroups showed similar location i. e, membranous expression ($p = 0.7716$).

Percentage – In early OSMF, 14 cases showed >50 % of positivity & 1 case showed 1–25 % positivity. In advanced OSMF, all cases (100 %) showed >50 % positivity rate ($p = 0.7716$).

3.2. Comparison of ZEB1 epithelial expression among histo-pathological grades of OSMF by mann whitney U test (Table 3) (Fig. 3)

Intensity – In early OSMF, 11 (73 %) cases showed mild & 4 (27 %) cases showed intense intensity. Whereas in advanced OSMF, a similar staining intensity was observed mild intensity. (p-value:0.8357).

Location – In early OSMF and advanced OSMF, almost equal cases showed cytoplasmic expression (12 cases) and 2 cases showed cytoplasmic and nuclear expression with no statistical difference in intensity (p-value:0.8035).

Percentage – In early OSMF, 6 cases showed >50 % of positivity, 6 cases showed 25–50 % positivity and 2 cases showed 1–25 % positivity and 1 case showed no expression. In advanced OSMF, 8 cases showed >50 % positivity rate, 4 cases showed 25–50 % and 3 cases showed

Tablec2
Comparison of E-Cadherin expression in Histo-pathological grades of OSMF by Mann-Whitney U test.

E-Cadherin Expression	Intensity				Location						Percentage of positivity				
	0	1	2	Z- Value	0	1	2	3	4	Z- Value	0	1	2	3	Z- Value
Early OSMF (n = 15)	0	5	10	−1.5347	0	14	0	1	0	0.2903	0	1	0	14	0.2903
Advanced OSMF (n = 15)	0	0	15	**P- Value** 0.1249	0	15	0	0	0	**p- Value** 0.7716	0	0	0	15	**p- Value** 0.7716

OSMF: oral submucous fibrosis Intensity: 0 = Negative, 1 = Mild, 2 = Moderate.
Location: 0 = Absent, 1 = Membrane, 2 = M + C, 3 = Cytoplasm 4 = Nuclear.
Percentage: 0 = Absent, 1 = 1–25 %, 2 = 25–50 %, 3= >50 %.

Table 3
Comparison of ZEB1 epithelial expression among histopathological grades of OSMF by Mann Whitney U test.

ZEB1 expression in epithelium	Intensity				Location						Percentage of positivity				
	0	1	2	Z- Value	0	1	2	3	4	Z- Value	0	1	2	3	Z- Value
Early OSMF (n = 15)	1	10	4	−0.2074	1	0	12	2	0	−0.2489	1	2	6	6	−0.5392
Advanced OSMF (n = 15)	0	11	4	**P- Value** 0.8357	0	0	13	2	0	**p- Value** 0.8035	0	3	4	8	**p- Value** 0.5897

Intensity: 0 = Negative, 1 = Mild, 2 = Moderate.
Location: 0 = Absent, 1 = Membrane, 2 = Cytoplasm, 3 = C + N, 4 = Nuclear.
Percentage: 0 = Absent, 1 = 1–25 %, 2 = 25–50 %, 3=>50 %.

Table 4
Comparison of ZEB1 connective tissue expression among OSMF grades using Mann Whitney *U* Test.

ZEB1 expression in connective tissue	Intensity				Percentage of positivity				
	0	1	2	Z- Value	0	1	2	3	Z- Value
Early OSMF (n = 15)	0	3	12	−0.6014	0	1	3	11	−0.9333
Advanced OSMF (n = 15)	0	1	14	**P- Value** 0.5476	0	0	1	14	**p- Value** 0.3507

Intensity: 0 = Negative, 1 = Mild, 2 = Moderate.
Location: 0 = Absent, 1 = Membrane, 2 = Cytoplasm, 3 = C + N, 4 = Nuclear.
Percentage: 0 = Absent, 1 = 1–25 %, 2 = 25–50 %, 3=>50 %.

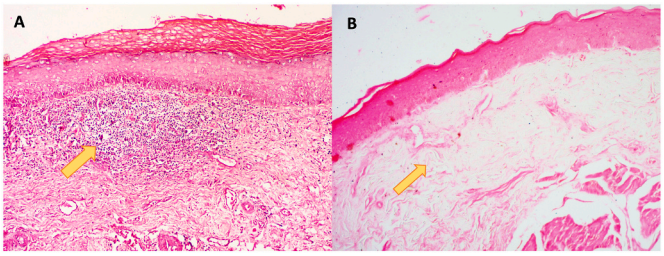

Fig. 1. A: Hematoxylin and Eosin-stained section of Early OSMF showing hyperkeratosis, atrophic epithelium and inflammatory infiltrate and fibrosis subepithelially (yellow arrow). B: Advanced OSMF with features of atrophic epithelium, dense fibrosis and compressed capillaries in the subepithelial component (yellow arrow) and shrunken lamina propria with muscles close to the epithelium.

1–25 % positivity rate. A slight increase in percentage of positivity from early to advanced OSMF was observed. Statistically significance was not obtained (p-value:0.7716).

3.3. Comparison of ZEB1 connective tissue expression among OSMF grades using mann –whitney U test (Table 4) (Fig. 3)

Intensity – In early OSMF, 3 (20 %) cases showed mild & 12 cases (80 %) showed intense intensity. Whereas in advanced OSMF, 14 (93 %) cases showed intense intensity and 1 (7 %) case showed mild intensity.

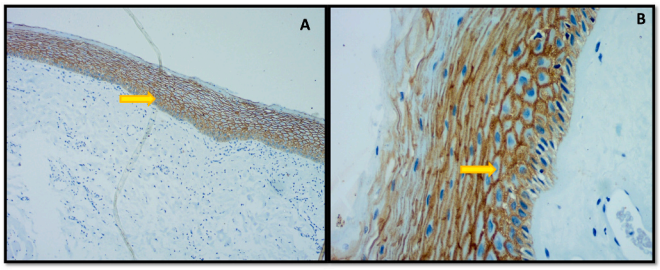

Fig. 2. A - Immuno-expression of E-cadherin in the epithelium (yellow arrow) is intense and membranous in Early OSMF (IHC, 10X) B: Immuno-expression of E-cadherin (yellow arrow) is intense and membranous in Advanced OSMF (IHC, 10X).

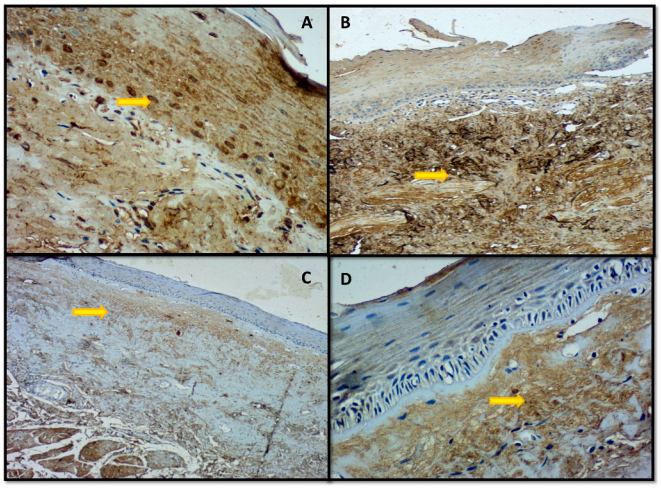

Fig. 3. A - Immuno-expression of ZEB1 in early OSMF epithelium is intense and is cytoplamsic as well as nuclear (yellow arrow). In connective tissue, it shows intense expression (yellow arrow). (IHC, 10X) B: Immuno-expression of ZEB1 in advanced OSMF epithelium is intense and is cytoplasmic. In connective tissue, it shows intense expression (yellow arrow). (IHC, 10X).

An increase in intensity from early to advanced OSMF was observed but no statistical significance was observed (p-value: 0.5476).

Percentage – In early OSMF, 11 cases (73 %) showed >50 % of positivity while in advanced OSMF, 14 cases (93 %) showed >50 % positivity rate. A slight increase in percentage of positivity in ZEB 1 connective tissue expression was noted from early to advanced OSMF (p value: 0.3507). An increase in intensity and percentage of positivity of ZEB 1 expression in connective tissue was observed in advanced OSMF as compared to Early OSMF.

4. Discussion

Transcription factors act synergistically to bring about the epithelial cell reprogramming and regulation of these factors controls the expression of critical markers of EMT.[26] Evidence suggests a cross talk between various signaling pathways and some studies suggest the inhibition of single transcription factor is enough to block EMT. EMT has detrimental role in the progression of fibrosis and cancer metastasis. A thorough understanding of signaling pathways involved in EMT and the tumor microenvironment in OSMF and OSCC paves way for newer strategies for management. Numerous invitro studies has shown association of markers such as E-Cadherin and ZEB1 with its involvement in OSMF and OSCC.[27] It showed the participation of up-regulated ZEB1 and down-regulated E-Cadherin markers with various EMT regulating pathways.[27] The present study thus evaluates this relationship at tissue level using immunohistochemistry.

E-Cadherin expression: In OSMF, the intensity, location, and percentage of positivity of E-cadherin expression did not alter significantly. Further, the intensity of E cadherin did not decrease in advanced OSMF as compared to early OSMF. However, studies indicate that as the OSMF advances, the level of E-cadherin expression declines and E-cadherin membranous loss in OSMF is reported in several publications.[28] The reason for this could be that OSMF's malignant transformation potential corresponds to its functional loss of epithelium and presence of dysplasia.[28] The loss of E-cadherin in the OSMF epithelium could indicate a disturbance with intercellular communication and indicates start of pro-carcinogenic signaling process in this epithelial layer.[28] This could be because OSMF epithelial cells may exhibit dysplasia, which contributes to its malignant transformation potential. The reduced E-cadherin membranous expression in the epithelium has an adverse effect on cellular adhesiveness, cellular differentiation, and cellular polarity, causing cells to acquire a motility, which is a crucial factor associated with malignant transformation.[28]

4.1. ZEB1 expression

In epithelium: ZEB1 epithelial expression in OSMF was mildly high, with the majority of cases demonstrating expression in the cytoplasm of epithelial cells. ZEB1 is the key transcription factor of EMT and facilitates EMT in a number of ways, one of which is through regulating target genes via its protein binding domains, especially that of E-Cadherin.[13,16] Its role in activating target genes involved in acquiring the mesenchymal phenotype is the most likely explanation for such an increase in expression and relocation into the cytoplasm.[16]

ZEB1 interacts with microRNAs (miRNAs) such as miR-200c and miR-205 to mediate multiple signaling pathways, such as wingless/integrated (Wnt), hippo pathway, TGF-β to regulate the biological processes of inflammation, fibrosis, tumor metastasis and proliferation. The most frequent mechanism by which ZEB 1induces EMT related fibrosis is by the activation of TGF-beta pathway.[29]

In Connective tissue: The stromal expression of ZEB1 reflects its normal expression in immune cells and/or a subset of fibroblasts. ZEB1 expression in the stroma is similar to that found in immune cells and/or a subpopulation of fibroblasts.[29] The presence of ZEB1 in these stromal cells could indicate that it has an impact on the tumor microenvironment. It helps to create the tumor microenvironment by controlling the amounts of inflammatory cytokines like IL-6/8.[29] Extracellular signals generated from the tumor microenvironment abnormally activate the EMT program in cancer cells. The EMT-promoting tumor microenvironment is made up of abnormally increased growth factors, inflammatory cytokines, and some intra-tumoral physical stressors like hypoxia. As a result, cancer cells have a collection of EMT transcriptional factors (EMT-TF) activated, allowing them to directly execute EMT programs. In malignant cells, myocyte enhancer factor 2D (MEF2D) may additionally acquire microenvironment cytokines including EGF, IGF2, and bFGF via the MAPK or PI3K pathway.[29] These are then translated into the ZEB1 transcriptional target genes. MEF2D is also an early responder gene to hypoxia, mediating hypoxia-induced ZEB1 expression as well as EMT.[29]

Increased ZEB1 expression in stroma was most prominent in OSMF. These findings were in accordance with the studies reported in the literature. According to Shetty et al. 2020,[30] ZEB1 is known to play a role in initiating myofibroblasts activity in "buccal mucosal fibroblasts (BMFs)" via its attachment to "promoter region" of alpha-SMA, leading to transdifferentiation of myofibroblasts and fibrosis via EMT.[27–30]

Hutchinson et al. used nuclear and cytoplasmic RNA fractions from human fibroblasts and lymphoblasts to identify LINC00084 (nuclear enriched autosomal transcript 1; NEAT1), a nuclear-retained lncRNA.[30] LINC00084 is ordinarily found in paraspeckles, but when stimulated by inflammation-activating signals, it dissociates from the nuclear bodies and translocates into the cytoplasm, promoting fibrosis in illness.[30] According to Lee et al., 2021, increased LINC00084 promotes myofibroblast activation by sponging miR-204, which could lead to an increase in ECM components and fibrosis.[31] In addition, according to a paper by Qian et al., 2019, ZEB1 plays a vital function in initiating fibrogenesis via TGF-beta signaling pathways.[30]

As a result, ZEB1 has been related to organ fibrosis, such as pulmonary and ocular fibrosis. These findings reveal ZEB1's role in OSMF as a strong inducer of fibrosis by activating Type 2 EMT and transdifferentiation of myofibroblasts.[27–30]

The increased ZEB 1 expression in the stroma of OSMF may represent abnormal healing reponse for microinjuries in the epithelium due to arecoline. The microinjuries predispose to activation of EGFR_ERK-RAS pathway via interaction with Smad 3 TGF beta pathway. This induces trans differentiation of fibroblasts into myofibroblasts via EMT by upregulation of ZEB1 that drives enhanced expression of α SMA. Occasionally, the arecoline can directly induce αSMA to bind with ZEB1 promoting region predisposing to EMT. This transdifferentiation of myofibroblast via ZEB1 mediated EMT may predispose to fibrosis in OSMF.[27–30]

Additionally, ZEB1 being a transcriptional repressor that negatively regulates E−cadherin expression as evidenced in OSCC[30] may predispose to EMT associated carcinogenesis especially in oral squamous cell carcinoma arising in the background of OSMF.

4.1.1. Strengths and limitations of the study

This study gives an insight into the role of epithelial mesenchymal transition in OSMF and highlight the importance of ZEB1 as a marker for EMT in OSMF. This is one of the few studies to evaluate these markers at tissue level using immunohistochemistry, to highlight the role of ZEB 1 in the pathogenesis of OSMF. The sample size in this study is relatively small and the results do not have confidence intervals. Further, we have not evaluated these markers in OSMF turning into OSCC which could have given a confirmatory insight into the malignant transformation potential. The results reported in this study need validation with larger sample size and conduct of multicentric studies could be the future scope of this study.

5. Conclusion

An increase in intensity and percentage of positivity of ZEB 1 expression in connective tissue was observed in advanced OSMF as

compared to Early OSMF. This may reflect a role of ZEB1 in mediating EMT via transdifferentiation of fibroblast into myofibroblast and thus predispose to fibrosis in OSMF. ZEB1 being a transcriptional repressor that negatively regulates E−cadherin expression may predispose to EMT associated carcinogenesis and malignant transformation in OSMF. There is a need for further extensive studies regarding the role of ZEB1 in fibrosis and malignancy seen in OSMF.

Ethical statement

The study has been approved by the Institutional Ethical Committee, KLE VK Institute of Dental Sciences, Belagavi with the Number 1321.

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Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material (data transparency)

The Data related to the study is available with the first author and will be provided if asked immediately.

Code availability (software application or custom code)

Not applicable for this study.

Author contribution statement

The conceptualization, investigation, validation, analysis, writing and review, supervision and project administration was done by Dr. Punnya Angadi.

The methodology, validation, investigation, validation, analysis, collection of resources, writing of draft was done by Dr Gouri P.

Consent to participate (include appropriate statements)

Not Applicable as this study has been done on archival tissue specimens available in the department.

Consent for publication (include appropriate statements)

All authors have read and approved the final version of the manuscript submitted.

References

1. Wollina U, Verma SB, Ali FM, Patil K. Oral submucous fibrosis: an update. *Clin Cosmet Invest Dermatol*. 2015;8:193–204.
2. Arakeri G, Rai KK, Hunasgi S, Merkx MAW, Gao S, Brennan PA. Oral submucous fibrosis: an update on current theories of pathogenesis. *J Oral Pathol Med*. 2017 Jul;46(6):406–412.
3. More CB, Rao NR. Proposed clinical definition for oral submucous fibrosis. *J Oral Biol Craniofac Res*. 2019;9(4):311–314.
4. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. *Oral Oncol*. 2006 Jul;42(6):561–568.
5. Rao NR, Villa A, More CB, Jayasinghe RD, Kerr AR, Johnson NW. Oral submucous fibrosis: a contemporary narrative review with a proposed inter-professional approach for an early diagnosis and clinical management. *J Otolaryngol Head Neck Surg*. 2020 Jan 8;49(1):3.
6. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, et al. Oral potentially malignant disorders: a consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. *Oral Dis*. 2021 Nov;27(8):1862–1880.
7. Gupta MK, Mhaske S, Ragavendra R. *Oral Submucous Fibrosis: Current Concepts in Etiopathogenesis*. vol. 1. 2008 Jul:39–44.
8. Tupkari JV, Bhavthankar JD, Mandate MS. Oral submucous fibrosis (OSMF): a study of 101 cases. *J Indian Acad Oral Med Radiol*. 2007 Apr 1;19(2):311.
9. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review) *Oncol Lett*. 2014;8(1):7–11.
10. Khowal S, Monga S, Naqvi SH, Jain SK, Wajid S. Molecular winnowing, expressional analyses and interactome scrutiny of cellular proteomes of oral squamous cell carcinoma. *Adv Cancer Biology-Metastasis*. 2021;2, 100003.
11. Bansal SK, Leekha S, Puri D. Biochemical changes in OSMF. *J Adv Med Dent Sci*. 2013;1(2):101–105.
12. Rajendran R, Vijayakumar T, Vasudevan DM. An alternative pathogenetic pathway for oral submucous fibrosis (OSMF). *Med Hypotheses*. 1989 Sep;30(1):35–37.
13. Arakeri G, Brennan PA. Oral submucous fibrosis: an overview of the aetiology, pathogenesis, classification, and principles of management. *Br J Oral Maxillofac Surg*. 2013 Oct;51(7):587–593.
14. Passi D, Bhanot P, Kacker D, Chahal D, Atri M, Panwar Y. Oral submucous fibrosis: newer proposed classification with critical updates in pathogenesis and management strategies. *Natl J Maxillofac Surg*. 2017 Jul-Dec;8(2):89–94.
15. Khan S, Chatra L, Prashanth SK, Veena KM, Rao PK. Pathogenesis of oral submucous fibrosis. *J Cancer Res Therapeut*. 2012 Apr-Jun;8(2):199–203.
16. Phulari RGS, Dave EJ. A systematic review on the mechanisms of malignant transformation of oral submucous fibrosis. *Eur J Cancer Prev*. 2020 Sep;29(5):470–473.
17. Chaudhary M, Bajaj S, Bohra S, Swastika N, Hande A. The domino effect: role of hypoxia in malignant transformation of oral submucous fibrosis. *J Oral Maxillofac Pathol*. 2015 May-Aug;19(2):122–127.
18. Ray JG, Ranganathan K, Chattopadhyay A. Malignant transformation of oral submucous fibrosis: overview of histopathological aspects. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016 Aug;122(2):200–209.
19. Ekanayaka RP, Tilakaratne WM. Oral submucous fibrosis: review on mechanisms of malignant transformation. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016 Aug;122(2):192–199.
20. Kujan O, Mello FW, Warnakulasuriya S. Malignant transformation of oral submucous fibrosis: a systematic review and meta-analysis. *Oral Dis*. 2021 Nov;27(8):1936–1946.
21. Goel S, Ahmed J, Singh MP, Nahar P. Oral submucous fibrosis: a clinico-histopathological comparative study in population of Southern Rajasthan. *J Carcinog Mutagen*. 2010;1(2), 108-1.
22. Motgi AA, Shete MV, Chavan MS, Diwaan NN, Sapkal R, Channe P. Assessment of correlation between clinical staging, functional staging, and histopathological grading of oral submucous fibrosis. *J Carcinog*. 2021 Oct 7;20:16.
23. Hande AH, Chaudhary MS, Gawande MN, et al. Oral submucous fibrosis: an enigmatic morpho-insight. *J Cancer Res Therapeut*. 2019 Jul-Sep;15(3):463–469.
24. Agarwal RK, Hebbale M, Mhapuskar A, Tepan M. Correlation of ultrasonographic measurements, histopathological grading, and clinical staging in oral submucous fibrosis. *Indian J Dent Res*. 2017 Sep-Oct;28(5):476–481.
25. Chaw SY, Abdul Majeed A, Dalley AJ, Chan A, Stein S, Farah CS. Epithelial to mesenchymal transition (EMT) biomarkers–E-cadherin, beta-catenin, APC and Vimentin–in oral squamous cell carcinogenesis and transformation. *Oral Oncol*. 2012 Oct;48(10):997–1006.
26. Costa LC, Leite CF, Cardoso SV, et al. Expression of epithelial-mesenchymal transition markers at the invasive front of oral squamous cell carcinoma. *J Appl Oral Sci*. 2015 Mar-Apr;23(2):169–178.
27. Pindborg JJ, Sirasat SM. Oral submucous fibrosis. *Oral Surg Oral Med Oral Pathol*. 1966;22:764–769.
28. Chang YC, Tsai CH, Lai YL, et al. Arecoline-induced myofibroblast transdifferentiation from human buccal mucosal fibroblasts is mediated by ZEB1. *J Cell Mol Med*. 2014 Apr;18(4):698–708.
29. Hosur MB, Puranik RS, Vanaki SS, Puranik SR, Sudhakara M, Das S. Evaluation of immunohistochemical expression of epithelial- mesenchymal transition markers E-cadherin, Twist and Snail in oral submucous fibrosis and their possible association with malignant transformation. *J Oral Maxillofac Pathol*. 2021 Jan-Apr;25(1):97–104.
30. Shetty SS, Sharma M, Fonseca FP, et al. Signaling pathways promoting epithelial mesenchymal transition in oral submucous fibrosis and oral squamous cell carcinoma. *Jpn Dent Sci Rev*. 2020 Nov;56(1):97–108.
31. Lee YH, Liao YW, Lu MY, Hsieh PL, Yu CC. LINC00084/miR-204/ZEB1 Axis mediates myofibroblastic differentiation activity in fibrotic buccal mucosa fibroblasts: therapeutic target for oral submucous fibrosis. *J Personalized Med*. 2021 Jul 23;11(8):707.