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# Bio Markers in Oral Submucosal Fibrosis

[1]Deepak Narang, Reader, Department of Oral Medicine and Radiology, Deshbhagat Dental College, Punjab, India.

[2]Tejveer Singh, Professor, Department of Oral and Maxillofacial surgery, Deshbhagat Dental College, Punjab, India.

[3]Prabhjot Kaur, Reader, Department of oral and maxillofacial Pathology, Deshbhagat Dental College, Punjab, India.

[4]Amandeep Singh, Professor & HOD, Department of Community Dentistry, Deshbhagat Dental College, Punjab, India.

[5]Arvind Arora, Professor, Department of Conservative Dentistry and Endodontics, Deshbhagat Dental College, Punjab, India.

**Corresponding Author:** Deepak Narang, Reader, Department of Oral Medicine and Radiology, Deshbhagat Dental College, Punjab, India.

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**Abstract**

Oral submucous fibrosis (OSF) is a collagen deposition condition that impairs oral function and quality of life in patients. It has the potential to develop into cancer. Based on clinical and biomolecular evidence, this review outlines the risk factors, pathogenic processes, and therapies for OSF. Chewing betel nuts is a key risk factor for OSF in Asia.

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To improve a patient's quality of life, treatments focus on lowering inflammation and enhancing mouth opening. Finally, high-quality clinical investigations are required to assist doctors in the development and application of molecular biomarkers and standard treatment guidelines.

**Keywords**: Biomarkers; Epidemiology; Oral Submucous Fibrosis (OSF);

## Introduction

Oral submucous fibrosis (OSF) is a chronic, progressive oral mucosal condition that can lead to cancer. The clinical manifestation of the disease is determined by its stage. Most patients initially experience a burning sensation or an aversion to spicy foods, as well as vesicles, particularly on the mouth. Ulceration and dry mouth are followed by fibrosis of the oral mucosa, which causes trismus by inducing rigidity of the lips, tongue, and palate[1].

The accumulation of thick collagen in the lamina propria, together with epithelial atrophy, are key histopathologic hallmarks. Juxta-epithelial inflammation

occurs first, followed by hyalinization. OSF is commonly observed in South-East Asian nations where areca nut chewing is common, implying that this practise is the most important etiological element in OSF pathophysiology. Importantly, over a 17-year period, the malignant transformation rate was found to be 7.6%[2].

Furthermore, because to the widespread usage of betel quid, more than 2400 new instances of oral squamous cell carcinoma (OSCC) developing from OSF are detected each year in Taiwan. As a result, early diagnosis of potentially malignant OSF is critical in preventing oral cancer. In OSF, numerous efforts have been done to investigate carcinogenesis and discover reliable diagnostic biomarkers[1].

However, no useful marker has yet been discovered. We used immunohistochemical labelling to look for indicators that could be beneficial in predicting the development of oral cancer in patients with OSF. Every biomarker has a unique attribution that must be chosen.

Cell proliferation can be assessed using Ki67 and cyclin D1. In head-and-neck squamous cell carcinomas, the tumour suppressor genes p16 and p53 were studied (HNSCCs). The incidence of p16 inactivation owing to mutation was reported to be 10% in HNSCCs, whereas the incidence of homozygous deletion was 33%[3].

Point mutations in the p53 gene are found in 10–17% of precancerous disease and 35–67% of OSCC. The expression levels of b-catenin and c-Jun were measured to further study the transcriptional activity in OSF tissue. The growth factor receptors c-Met and insulin-like growth factor II mRNA-binding protein 3 (IMP3) have been linked to tumour invasion.

Our goal was to create a risk assessment prediction model employing a combination of biomarkers. Although no single biomarker has been found to be effective in predicting carcinomatous transformation of potentially malignant OSF, the combined biomarkers identified in this study may be clinically useful in detecting high-risk OSF and reducing the incidence of OSCC, thereby assisting clinicians in making diagnosis, treatment, and prevention decisions[2].

**Biomarkers of Oral Sub Mucosal Fibrosis**

Normal, oral, and possibly malignant diseases, as well as early and late cancer stages, have certain underlying biological variations. Biomarkers for OSF have been established in recent years using existing biology techniques, including cytological characteristics, promoter methylation, polymorphism, mRNAs, microRNAs, non-coding RNAs, protein and trace elements in a solid biopsy, liquid biopsy from serum, and saliva[4].

The advantages of using cytology, tissue, serum, and saliva samples for analysis are numerous. With the right staining procedures, collecting and observing mucosal cells is simple. Histology is the most reliable type of inspection, but it is also the most intrusive, with long wait periods for test results and low patient approval. Body fluid biopsy testing has been developed, showing reduced invasiveness, shorter testing report turnaround times, and high patient acceptance, albeit more supporting data is needed to confirm accuracy[5].

Currently, traditional solid biopsy studies contain more biomarkers than liquid biopsy studies. In OSF stage classification, these biomarkers have not been frequently employed. To determine the accuracy of most novel biomarkers, larger sample sizes are required. The mainstay of OSF diagnosis is still clinical symptoms and pathological evaluation. In OSF diagnosis, combining instruments with biomarkers is a more effective way to save time and reagents[6].

Single or multiple biomarker expression levels may be employed as a new OSF staging approach for improving OSF evaluation index, evaluating OSF transformation to malignant tumour index, and for gene and targeted therapy, in addition to diagnosing OSF.

**Tumor Markers in Blood & Urine**

***Alpha-1 chymotrypsin*** and ***Factor XIIIa*** antibodies are specific markers for histiocyte and macrophages and are used for giant cell lesions, since they have been found to arise from precursors cells that express these markers[18].

***p53*** inactivation by ***MDM2 (Murine Double Minute)*** expression may occur in the pathogenesis of oral submucosal fibrosis.

***Bcl-2*** is used as a prognostic indicator in early oral squamous cell carcinoma. Decrease in CD-80 expression serves as marker for increased tumorigenicity during early Oral sub mucosal fibrosis[19].

***Cytokeratins- CK19 and CK8*** are markers of progressive premalignant changes in oral sub mucosal fibrosis. The aberrations of ***C-erb-1 and C-erb-2*** are indicators of early changes during carcinogenesis process in oral premalignant lesions[20].

Expression of cell cycle proteins ***p16 and p53*** along with ***Ki-67*** can be used as markers to identify evolution of oral precancerous disease and improves the identification of the degree of dysplasia[20].

Immunohistochemical ***p53*** over expression is valuable marker for early malignant transformation. Over expression of p53 along with PCNA is presumed predictors for malignant transformation of oral sub mucosal fibrosis.

***Beta-2 microglobulin*** was increased in oral submucous fibrosis (OSF) and oral cancer Survivin expression levels were higher in OSCC transformed from OSF[5]

***Ameloblastin (AMBN)*** gene mutations are responsible for the tumorigenesis of epithelial odontogenic tumours without Odontogenic ectomesenchyme. Reduced ameloblasts in the odontoma displayed most intense amelogenin expression.

***Calretinin*** is a calcium binding protein and is primarily expressed in central and peripheral nervous system and it is used as the diagnostic marker for oral submucosal fibrosis[20].

**Recent Advancement**

Recent epidemiological data indicate that the OSF prevalence has increased since 2000. One study recognized that OSCC originating from OSF is clinically more invasive and also exhibits a higher metastasis and recurrence rate than OSCC not originating from OSF (16). Therefore, there has been much focus on investigating biomarkers for the prevention and early detection of carcinomatous transformation[7].

**Types of bio markers in oral sub mucosal fibrosis**

In this review, we researched about several biomarkers to investigate the useful biomarkers in predicting oral cancer development in patients with OSF. The selection of biomarkers was based on the understanding of the physiological/pathological features of OSF tissue. We have observed that Ki67, cyclin D1, c-Met, IMP3, and b-catenin were found to show significantly different expression between the NOM and the OSF. All of these biomarkers have gained attention as prognostic biomarkers to predict the proliferation activity and transcriptional activity, as indicators for metastasis and poor prognosis and as good targets for therapeutic inhibition. Ki67 and cyclin D1 were used to evaluate the cell proliferation. Ki67 is a nuclear marker expressed in all phases of the cell cycle other than the G0 phase and is

widely used as a surrogate marker for proliferation in tumor samples[8].

Cyclin D1 modulates cell cycle transition from G1 to S phase and also plays a role in apoptosis (19). In our study, proliferating activity was found to be reduced in OSF samples compared with the NOM through the expression of cyclin D1 and Ki67. Contradictory results to our study were found by the research group of Ranganathan and Kavitha[9]

observing that Ki67 expression in the OSF was significantly higher than that of NOM, but less than that of OSCC. This different result may be explained as the proliferating activity of the OSF being largely dependent on the developmental stage. The OSF at the early stage may show a lower proliferating activity, as evidenced by atrophic epithelium. In our study, the proliferation activity of OSF samples with cancerous transformation showed higher proliferating activity than that of the NOM and OSF samples without transformation, suggesting that the switch toward the upregulation of the cell cycle from atrophic epithelium could represent transformation. Recent studies have shown that IMP3 is an important protein for tumor cell proliferation and invasion, indicating that IMP3 is an oncofetal protein that may play a critical role in malignant transformation and tumor progression[10].

IMP3 was found to be expressed in carcinoma lesions and oral leukoplakia with dysplasia (22). Previous studies have suggested that the IMP3 staining pattern should be a useful adjunct in distinguishing benign from malignant squamous epithelia. In our study, IMP3 expression increased in the OSF compared with the NOM, whereas we could not find a clear relationship with malignant transformation or epithelial dysplasia[11].

c-Met plays a crucial role in morphogenic organization during embryonic development and in the control of the structure and function of adult tissues, including cell migration and proliferation necessary for injury repair. Specifically, a significant increase in the expression of c-Met was noted in the transformation from NOM to epithelial dysplasia and to OSCC[12].

Notable biomarkers in our present research were Ki67 and p16, which showed significantly different expression between the transformation and non-transformation OSFs. According to the discriminant analysis, the combination of Ki67 and p16 was identified to show the highest predictability for high-risk OSF. Co-expression of both proteins was determined as the combined biomarker model ($P = 0.00108$)[13].

Accordingly, we propose that these two markers may play a role in predicting malignant transformation and could be used as biomarkers for high-risk OSF. p16 represents a negative regulator of the cell cycle that ensures the control of the cellular passage from G1 phase to S phase. Mitogenic stimuli, as well as growth factors, determine the activation of cyclin D, resulting in retinoblastoma protein phosphorylation, followed by the release of a transcription factor that ensures cell proliferation[14].

The relationship between p16 expression and its biologic behavior has been debatable, although the relationship between human papillomavirus infection and p16 expression has been highly correlated. In our study, we demonstrated that p16 positivity could provide evidence fo assessing high-risk OSF. Taken together, the proliferating activity of more than 8% and p16 expression of more than 5% can serve as promising biomarkers to assess the high risk of OSF. Furthermore, the proposed formula and cutoff value is the first trial to

predict risk assessment using combined biomarkers in potentially malignant OSF[15,16,17].

With the identification of high-risk patients with OSF, more intensive treatment modalities can be developed, resulting in a reduced incidence of OSCC[18,19]. The positive relationship between epithelial dysplasia and malignant transformation in OSF has been investigated. [20]

**Conclusion**

The prevalence of OSF and the rate of malignant transformation are different among countries. Quitting betel nut chewing is the best strategy to prevent OSF and potential malignancy. Regardless of the strategy, clinical diagnosis and treatment are still based on conservative methods. The treatment must improve the elasticity of the oral mucosa and mouth opening distance. This ensures that patients have normal oral functions like speaking and eating to improves the patient's quality of life and provides an adequate nutritional intake. High-quality clinical studies are needed to help clinicians to develop and apply molecular biomarkers and to formulate standard treatment guidelines for OSF

**References**


1. Dionne, K.R.; Warnakulasuriya, S.; Zain, R.B.; Cheong, S.C. Potentially malignant disorders of the oral cavity: Current practice and future directions in the clinic and laboratory. Int. J. Cancer 2015, 136, 503–515. [CrossRef] [PubMed]
2. Chole, R.H.; Gondivkar, S.M.; Gadbail, A.R.; Balsaraf, S.; Chaudhary, S.; Dhore, S.V.; Ghonmode, S.; Balwani, S.; Mankar, M.; Tiwari, M.; et al. Review of drug treatment of oral submucous fibrosis. Oral Oncol. 2012, 48, 393–398. [CrossRef] [PubMed]
3. Wang, Y.Y.; Tail, Y.H.; Wang, W.C.; Chen, C.Y.; Kao, Y.H.; Chen, Y.K.; Chen, C.H. Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders. BMC Oral Health 2014, 14, 99. [CrossRef] [PubMed]
4. Zhang, X.; Reichart, P.A. A review of betel quid chewing, oral cancer and precancer in Mainland China. Oral Oncol. 2007, 43, 424–430. [CrossRef] [PubMed]
5. Tilakaratne, W.M.; Ekanayaka, R.P.; Warnakulasuriya, S. Oral submucous fibrosis: A historical perspective and a review on etiology and pathogenesis. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 2016, 122, 178–191. [CrossRef]
6. Chattopadhyay, A.; Ray, J.G. Molecular Pathology of Malignant Transformation of Oral Submucous Fibrosis. J. Environ. Pathol. Toxicol. Oncol. 2016, 35, 193–205. [CrossRef]
7. Chang, M.C.; Chiang, C.P.; Lin, C.L.; Lee, J.J.; Hahn, L.J.; Jeng, J.H. Cell-mediated immunity and head and neck cancer: With special emphasis on betel quid chewing habit. Oral Oncol. 2005, 41, 757–775. [CrossRef]
8. Cox, S.C.; Walker, D.M. Oral submucous fibrosis. A review. Aust. Dent. J. 1996, 41, 294–299. [CrossRef]
9. Liu, B.; Shen, M.; Xiong, J.; Yuan, Y.; Wu, X.; Gao, X.; Xu, J.; Guo, F.; Jian, X. Synergistic effects of betel quid chewing, tobacco use (in the form of cigarette smoking), and alcohol consumption on the risk of malignant transformation of oral submucous fibrosis (OSF): A case-control study in Hunan Province, China. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 2015, 120, 337–345. [CrossRef]

10. Reichart, P.A.; Nguyen, X.H. Betel quid chewing, oral cancer and other oral mucosal diseases in Vietnam: A review. J. Oral Pathol. Med. Off. Publ. Int. Assoc. Oral Pathol. Am. Acad. Oral Pathol. 2008, 37, 511–514. [CrossRef]
11. Yang, S.F.; Wang, Y.H.; Su, N.Y.; Yu, H.C.; Wei, C.Y.; Yu, C.H.; Chang, Y.C. Changes in prevalence of precancerous oral submucous fibrosis from 1996 to 2013 in Taiwan: A nationwide population-based retrospective study. J. Med. Assoc. 2018, 117, 147–152. [CrossRef] [PubMed]
12. Nigam, N.K.; Aravinda, K.; Dhillon, M.; Gupta, S.; Reddy, S.; Srinivas Raju, M. Prevalence of oral submucous fibrosis among habitual gutkha and areca nut chewers in Moradabad district. J. Oral Biol. Craniofac. Res. 2014, 4, 8–13. [CrossRef] [PubMed]
13. Gottipamula, S.; Sundarrajan, S.; Moorthy, A.; Padmanabhan, S.; Sridhar, K.N. Buccal Mucosal Epithelial Cells Downregulate CTGF Expression in Buccal Submucosal Fibrosis Fibroblasts. J. Maxillofac. Oral Surg. 2018, 17, 254–259. [CrossRef] [PubMed]
14. Maher, R.; Lee, A.J.; Warnakulasuriya, K.A.; Lewis, J.A.; Johnson, N.W. Role of areca nut in the causation of oral submucous fibrosis: A case-control study in Pakistan. J. Oral Pathol. Med. Off. Publ. Int. Assoc. Oral Pathol. Am. Acad. Oral Pathol. 1994, 23, 65–69. [CrossRef] [PubMed]
15. Pindborg, J.J.; Murti, P.R.; Bhonsle, R.B.; Gupta, P.C.; Daftary, D.K.; Mehta, F.S. Oral submucous fibrosis as a precancerous condition. Scand. J. Dent. Res. 1984, 92, 224–229. [CrossRef] [PubMed]
16. Murti, P.R.; Bhonsle, R.B.; Pindborg, J.J.; Daftary, D.K.; Gupta, P.C.; Mehta, F.S. Malignant transformation rate in oral submucous fibrosis over a 17-year period. Community Dent. Oral Epidemiol. 1985, 13, 340–341. [CrossRef]
17. Shiau, Y.Y.; Kwan, H.W. Submucous fibrosis in Taiwan. Oral Surg. Oral Med. Oral Pathol. 1979, 47, 453–457. [CrossRef]
18. Tang, J.G.; Jian, X.F.; Gao, M.L.; Ling, T.Y.; Zhang, K.H. Epidemiological survey of oral submucous fibrosis in Xiangtan City, Hunan Province, China. Community Dent. Oral Epidemiol. 1997, 25, 177–180. [CrossRef]
19. Lian Ie, B.; Tseng, Y.T.; Su, C.C.; Tsai, K.Y. Progression of precancerous lesions to oral cancer: Results based on the Taiwan National Health Insurance Database. Oral Oncol. 2013, 49, 427–430. [CrossRef]
20. Yang, P.Y.; Chen, Y.T.; Wang, Y.H.; Su, N.Y.; Yu, H.C.; Chang, Y.C. Malignant transformation of oral submucous fibrosis in Taiwan: A nationwide population-based retrospective cohort study. J. Oral Pathol. Med. Off. Publ. Int. Assoc. Oral Pathol. Am. Acad. Oral Pathol. 2017, 46, 1040–1045. [CrossRef]