REVIEW ARTICLE

# ORAL SUBMUCOUS FIBROSIS PAST AND RECENT CONCEPTS IN ETIOPATHOGENESIS


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**ABSTRACT**

Oral submucous fibrosis (OSF) is a premalignant condition, affecting the people of South East Asia. The understanding of the exact role of etiological agents like areca nut with respect to pathogenesis will help in the management and treatment modalities of this condition. This article provides an overview of the etiopathogenesis with emphasis on the recent concepts related to this chronic Premalignant Condition.



**Key Words:** Areca nut, Arecoline, Oral precancer, Oral submucous fibrosis.

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## INTRODUCTION

Oral submucous fibrosis (OSF), is a disease associated with the chewing of areca nut, an ingredient of betel quid, and is prevalent in South East Asian populations. Pindborg in 1966 defined OSF as “an insidious chronic disease affecting any part of the oral cavity and sometimes pharynx, although occasionally preceded by and\or associated with vesicle formation, it is always associated with Juxtaepithelial inflammatory reaction followed by fibro elastic changes in the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa causing trismus and difficulty in eating”.[1] OSF has a propensity for malignant transformation. The association of betel quid chewing, OSF, and oral squamous cell carcinoma is quite profound, especially in Taiwan and the Indian subcontinent where up to 80% of oral squamous cell carcinoma is associated with the habit. Epidemiological studies have shown that the rate of malignant transformation ranges from 3 to 17%.

**Etiology:**

Epidemiological data and intervention studies suggest that areca nut is the main aetiological factor for OSF.[2-8]

**Areca nut:**

The term areca nut is used to denote the unhusked whole fruit of the areca nut tree and term betel nut is used exclusively to refer to the inner kernel or seed which is obtained after removing husk. The betel nut has psychotropic and anti helminthic property due to presence of areca alkaloids. Four alkaloids have been conclusively identified in biochemical studies-

arecoline, arecaidine, guvacine & guvacoline, of which arecoline is the main agent. These alkaloids have powerful parasympathetic properties which produce euphoria and counteract fatigue. Nitrosation of arecoline leads to the formation of areca nut specific nitrosamine namely nitrosoguvacoline, nitrosoguvacine and 3-methyl nitrosominopropionitrile, which alkylate DNA. These alkaloids undergo nitrosation and give rise to N-Nitrosamine, which might have cytotoxic effect on cells.[9]

**Areca and slaked lime:**

In a habitual betel nut chewer, oral submucous fibrosis may be caused by the amount of tannic acid contained in the betel nut, the influence of mixed calcium powder and the conditional action of arecoline content in betel nut, affecting the vascular supply of oral mucosa and causing neurotropic disorder. This view was further supported by the finding that, addition of slaked lime Ca(OH)2 to areca nut in pan facilitates hydrolysis of arecoline to arecaidine (more potent than arecoline) making this agent available in the oral environment.

The role of other etiological agents and factors so far studied are:

- Chillies
- Genetic predisposition
- Carcinogenicity of tobacco & areca nut
- Immunologic factors
- Nutritional factors
- Autoimmune process
- Su (1954) attributed it to the tannic acid and arecoline contents of betel nut, together with influence of lime.[10]
- Rao (1962) linked it to collegeno- pathies.[11]
- Sirsat and khanolkar(1962)attributed it to irritation caused by capsaicin.[12]
- Abrol and Krishnamoorthy(1970) suggested a genetic predisposition with supra added local irritation from betel nut, chillies, spices and condiments.[13]
- Ramanathan.K (1981) is of the view that OSF is an asian version of Sideropenic dysphagia. He suggested that OSF appears to be an altered oral mucosa following a prolonged deficiency of iron or vitamin B complex, especially folic acid. This altered oral mucosa subsequently develops hypersensitivity to oral irritants such as spices especially chillies and betel quid.[14]
- W.M. Tilakaratne (2005) depicted the role of Autoimmunity as an aetiological factor.

The reasons for investigating an autoimmune basis, included, slight female predilection and occurrence in the middle age reported in some studies, the presence of circulating immune complexes, their immunoglobulin contents and the detection of various autoantibodies in patient's sera. Increased levels of immune complexes and raised serum levels of IgG, IgA and IgM when compared with control groups have also been reported.[15]

**Molecular Pathogenesis:**

Rajalalitha P, Vali S[16] Reviewed and explained the molecular pathogenesis of Oral

submucous fibrosis as follows:

1. Collagen Production Pathway
2. Collagen Degradation Pathway

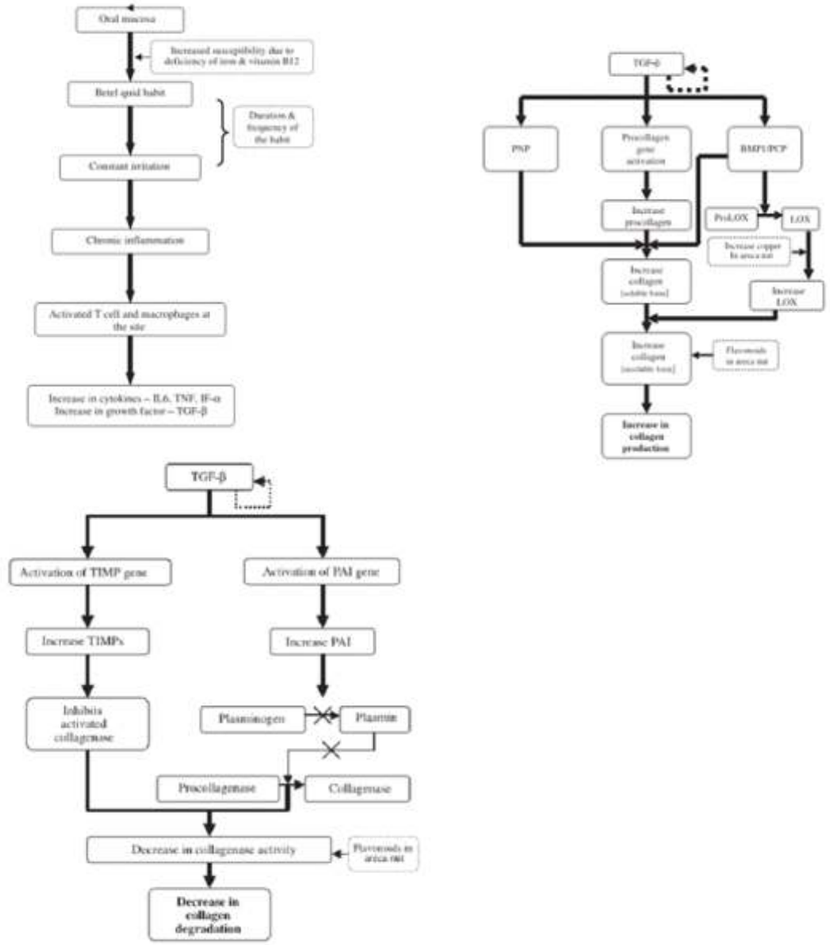


*Figure 1 Collagen degradation*

**1. Collagen Production Pathway**

It is regulated by TGF-beta which has autocrine activity. This activates pro-collagen genes, resulting in more production of pro-collagen. It also induces the secretion of pro-collagen proteinase (PCP) and pro-collagen N-proteinase (PNP), both of which are required for the conversion of pro-collagen to collagen fibrils. In Oral submucous fibrosis (OSF), there is increased cross - linking of collagen, resulting in increased insoluble form.

**Role of LOX**

This is facilitated by the increased activity and production of a key enzyme Lysyl Oxidase (LOX). It is an essential enzyme for final processing of

collagen fibers in to a stabilized covalently cross - linked mature fibrillar form that is resistant to proteolysis. LOX is dependent on copper for functional activity. Pro-collagen proteinase, bone morphogenetic protein 1, increased copper and flavanoids in betel quid stimulate LOX activity. Increased levels of LOX and its activity will cause increased cross -linking of collagen fibres, tilting the balance towards a fibrotic condition.

**2. Collagen degradation pathway:**

Two main events are modulated by TGF-beta, which decreases the collagen degradation:

a. Activation of tissue inhibitor of matrix metalloproteinase gene (TIMPs)

b. Activation of plasminogen activator (PAI) gene TGF-β activates the genes for TIMPs; there by more TIMP is formed. This inhibits the activated collagenase enzyme that is necessary for degradation of collagen. It also activates the gene for PAI, which is the inhibitor of plasminogen activator, thus there is no plasmin formation. Plasmin is required for the conversion of pro-collagenase and absence of plasmin results in absence of active collagenase. Along with this, flavanoids present in areca nut also inhibits the collagenase activity. The inhibition of the existing collagenase and decreased generation of active collagenase together results in a marked decrease in collagen degradation and resultant build up of collagen in OSMF

## Role of other factors in Pathogenesis:

**Role of of cyclo-oxygenase (COX-2)**

Treatment of the buccal mucosal fibroblasts with 80 µg/ml arecoline in culture revealed that COX-2 expression was up-regulated as early as half an hour, indicating this to be an early cellular response to arecoline at transcriptional level.[9]This indicates that COX-2 may play role in pathogenesis.[17]

**Role of Heat shock proteins (HSP)**

HSP 47, is a 47 kDa collagen-binding heat shock protein (HSP), which belongs to the serine protease inhibitor (serpin) super family containing a serpin signature sequence. HSP47 mRNA was up regulated by arecoline in human BMFs. Thus, the accumulation of collagen in oral mucosal connective tissue may be due to the synthesis of HSP 47 expression by resident cells in response to areca nut chewing.[18]

**Role of NF-kappa B**

NF-kappa B expression was significantly higher in OSF specimens and expressed mainly by fibroblasts, endothelial cells, and inflammatory cells. Safrole was cytotoxic to BMFs in a dose-dependent manner .Western blot demonstrated highly elevated NF-kappa B protein expression in BMFs stimulated by safrole. In addition, pre-treatment with pharmacological agents markedly inhibited the safrole induced-NF-kappa B expression. The result suggests that chewing areca quid may activate NF-kappa B expression that may be involved in the pathogenesis of OSF. NF-kappa B expression induced by safrole in fibroblasts may be mediated by ERK activation and COX-2 signal transduction pathway.[19]

**Role of S100A4 Expression**

S100A4, a member of the calcium-binding proteins, is dramatically elevated in a variety of fibrotic diseases. The critical role of S100A4 expression in the pathogenesis of OSF both in vitro and in vivo was accessed. S100A4 expression was significantly up-regulated in OSF specimens. Arecoline-induced S100A4 expression was down-regulated by rapamycin, PD98059, and Bay117082.Findings suggested that targeting S100A4 might be a potential therapeutic target for OSF through TIMP1/MMP9 down-regulation.[20]

**Role of basic fibroblastic growth factor (bFGF)**

The bFGF may either directly stimulate endothelial cell proliferation or facilitate Vascular endothelial growth factor (VEGF) endothelial cell interaction through the modulation of endothelial cell integrin or VEGF receptor expression. Endothelial cell derived IL-1 and bFGF modulate fibroblast properties independently, which supports the hypothesis that altered endothelial cell–fibroblast communication may be involved in the pathogenesis of OSMF.[21]

**Role of Lipids**

A significant decrease in plasma total cholesterol, High Density Lipoprotein (HDL), and triglycerides was observed in OSMF patients. Low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the rapidly dividing cells in malignancies. Several prospective and retrospective studies have shown an inverse trend between lower serum cholesterol and head and neck cancer. The decrease in total cholesterol in patients with OSF could be due to the greater utilization of lipids including total cholesterol by the cells for new membrane biogenesis.[22]

**Role of Mast cell**

Mast Cell Density (MCD) and Micro Vascular Density (MVD) in different grades of OSMF were assessed immunohistochemically and the results showed that when MCD increases there is an exponential increase in MVD proving that lesion is characterized by progressive fibrosis in early stages and there is a failure of degradation or remodelling in the advanced stages.[17] Interleukin-1 from the mast cells could cause increased fibroblastic response and mast cell derived tryptase causes increased production of type-I collagen and fibronectin thereby attributing to the increased fibrosis-in-OSF.[23]

**Role of minerals**

Significantly lower levels of hemoglobin and serum iron have been reported in OSMF by many authors.[24-27] In iron deficiency state, levels of cytochrome oxidase are low, consequently leading to epithelial atrophy. An atrophic epithelium makes the oral mucosa vulnerable to the soluble irritants. Further, lack of iron in tissues causes improper vascular channel formation resulting in decreased vascularity. This leads to derangement in the inflammatory reparative response of the lamina propria resulting in defective healing and scarification. Thus, the cumulative effect of these initiating and promoting factors leads to further fibrosis, which is a hallmark of OSMF.

Levels of circulating immune complexes (CIC), trace elements (copper, iron and selenium) in serum revealed increased circulating immune complex levels in the precancer and cancer patients. Serum copper levels showed gradual increase from precancer to cancer patients. However, serum iron levels were decreased significantly in the cancer group. Selenium levels showed marked decrease in the cancer group. Serum zinc levels are decreased in patients with OSMF which can act as indicator for malignant transformation.[28]

**Increased expression of fibrogenic cytokines:**

The most important finding in the various studies was the demonstration of increased expression of fibrogenic cytokines namely TGF α-1, PDGF and bFGF in OSF tissues compared to normal. These observations may suggest that the disease process in OSF may be an altered version of wound healing as recent findings show that the expression of various Extracellular molecules are similar to those seen in maturation of granulation tissue.[29]

**Genetic polymorphisms predisposing to OSF**

Polymorphisms of the genes coding for TNF-α has been reported as a significant risk factor for OSF. TNF-α is known to stimulate fibroblastic proliferation in vitro (Vilcek et al, 1986). Evidences suggest that collagen-related genes are altered due to ingredients in the quid. The genes COL1A2, COL3A1, COL6A1, COL6A3 and COL7A1 have been identified as definiteTGF targets and induced in fibroblasts at early stages of the disease. The transcriptional activation of these procollagen genes by TGF- β suggests that it may contribute to increased collagen levels in OSF.[30]

**Role of Transglutaminase-2**

Transglutaminase-2 (TGM-2) stabilizes extracellular matrix (ECM) proteins by cross-linking and has been implicated in several fibrotic disorders. The expression of TGM-2 was studied in OSMF tissues by real-time RT-PCR analysis, and significant over-expression was observed in most OSMF tissues when compared with normal tissues. Arecoline induced TGM-2 mRNA and protein expression as well as TGM-2 activity in human gingival fibroblast cells. The addition of methocramine hemihydrate (M-2 muscarinic acetylcholine receptor selective antagonist) or 8'-bromo-cAMP abolished arecoline-mediated TGM-

2 induction, suggesting a role for M-2 muscarinic acid receptor and a repressor role for cAMP. The study provided an evidence for TGM-2 over-expression in OSF and its regulation by arecoline in oral fibroblasts.[31]

**Role of PTEN immunoexpression**

PTEN, Phosphatase and tensin homolog (PTEN) a known tumor suppressor gene is mutated in a majority of human cancers and has also been implicated in several fibrotic disorders. The expression of PTEN in OSF and Oral squamous cell carcinoma were accessed to see if it had any role with the pathogenesis and malignant transformation of OSF. Data suggest that there is a significant loss of PTEN expression in OSF as compared to normal oral mucosa and that this trend increased from OSF to OSCC. Thus, alteration of PTEN is likely an important molecular event in OSF pathogenesis and oral carcinogenesis.[32]

OSF AND MALIGNANT-TRANSFORMATION

The precancerous nature of OSF was first described by Paymaster in 1956, when he studied slow growing squamous cell carcinoma in one third of the patients with the disease. This was confirmed by various groups and put forward five criteria to prove that the disease is precancerous. They included, high occurrence of OSF in oral cancer patients, higher incidence of squamous cell carcinoma in patients with OSF, histological diagnosis of cancer without any clinical suspicion in OSF, high frequency of epithelial dysplasia and higher prevalence of leukoplakia among OSF cases.

According to the current awareness of the disease and some refined criteria for grading dysplasia, it is reasonable to assume that the prevalence of dysplasia is more towards the midway of the reported range. Malignant transformation rate of OSF was found to be in the range of 7-13%.

Recently, the carcinogenic potential of areca nut without tobacco has been identified. The strong association of areca nut with OSF, its dose dependent effects and the confirmation of OSF as a potentially malignant disease leading to oral cancer provided further evidence for this assertion.

A review article on the pathogenesis of OSF hypothesized that dense fibrosis and less vascularity of the corium, in the presence of an altered cytokine activity creates a unique environment for carcinogens from both tobacco and areca nut to act on the epithelium. The authors have assumed that carcinogens from areca nut accumulate over a long period of time either on or immediately below the epithelium allowing the carcinogens to act for a longer duration before it diffuses into deeper tissues. Less vascularity may deny the quick absorption of carcinogens into the systemic circulation.

A study was conducted over a one year period which included 58 patients of OSF. On observation 15 (25.86%) patients showed mild dysplasia, 3 (5.17%) moderate and 2 (3.45%) severe.[33]

**CONCLUSION**

The current evidence suggests that arecoline in the areca nut is the key factor in initiating the disease process. The most ironical aspect of this condition is the lack of appropriate treatment modalities. Unlike tobacco pouch keratosis, oral submucous fibrosis does not regress with habit cessation, although mild cases may be treated with intra-lesional corticosteroids to reduce the symptoms. Dentists can play an important role in both education of patients about the perils of chewing betel quid and in the early diagnosis of high- risk premalignant conditions and cancer. Although the above mechanisms may explain the induction, maintenance and progression of fibrosis in OSF, further research is required in order to identify the mechanism leading to carcinogenesis in this fibrotic oral mucosa.

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