



King's Research Portal

DOI:

[10.1016/j.oooo.2016.04.003](https://doi.org/10.1016/j.oooo.2016.04.003)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Tilakaratne, W. M., Ekanayaka, R. P., & Warnakulasuriya, K. (2016). Oral submucous fibrosis: A historical perspective and a review on aetiology and pathogenesis. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics*. <https://doi.org/10.1016/j.oooo.2016.04.003>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

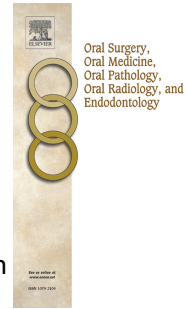
Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

Oral submucous fibrosis: A historical perspective and a review on aetiology and pathogenesis

Wanninayake Mudiyanseelage Tilakaratne, Rasika Priyadharshani Ekanayaka, Saman Warnakulasuriya



PII: S2212-4403(16)30018-9

DOI: [10.1016/j.oooo.2016.04.003](https://doi.org/10.1016/j.oooo.2016.04.003)

Reference: OOOO 1476

To appear in: *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*

Received Date: 2 June 2015

Revised Date: 22 March 2016

Accepted Date: 4 April 2016

Please cite this article as: Tilakaratne WM, Ekanayaka RP, Warnakulasuriya S, Oral submucous fibrosis: A historical perspective and a review on aetiology and pathogenesis, *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology* (2016), doi: 10.1016/j.oooo.2016.04.003.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Oral submucous fibrosis : A historical perspective and a review on aetiology and pathogenesis

Wanninayake Mudiyanseelage Tilakaratne¹, Rasika Priyadharshani Ekanayaka² Saman Warnakulasuriya³

^{1,2}Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka

³Department of Oral Medicine, King's College, London, UK and the WHO Collaborating Centre for Oral Cancer UK

Correspondence:

Saman Warnakulasuriya BDS, FDSRCS, PhD, DSc

Professor of Oral Medicine & Experimental Pathology

King's College London, London, SE5 9RS, UK

Phone: 00 44 20 3 299 2430

Fax: 00 44 20 3 299 3624

Email: s.warne@kcl.ac.uk

Oral submucous fibrosis : A historical perspective and a review on aetiology and pathogenesis

Wanninayake Mudiyanseilage Tilakaratne¹, Rasika Priyadharshani Ekanayaka¹, Saman Warnakulasuriya²

¹Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka

²Department of Oral Medicine, King's College, London, UK and the WHO Collaborating Centre for Oral Cancer, UK

Abstract

Background: Oral submucous fibrosis (OSF) is a chronic, insidious disease characterized by progressive submucosal fibrosis of the oral cavity and the oropharynx. People affected by this disease mostly live in south Asia, but migrants from these countries to the USA and Europe may present with OSF.

Purpose: We provide a historical background of the disease and the objective of this review is to update the current knowledge on the aetiology and aetiopathogenesis of OSF.

Introduction

Oral submucous fibrosis (OSF) is a chronic, insidious disease that affects the lamina propria of the oral mucosa and as the disease advances it involves tissues deeper in the submucosa of the oral cavity with resulting loss of fibroelasticity.¹ OSF is within a group of conditions that is classified under oral potentially malignant disorders². The disease manifests with blanching and stiffening of the oral mucosa leading to limitation in opening of the mouth. The presence of fibrous bands in lips, cheeks and soft palate is a hallmark of the disease. The disease is

histopathologically characterized by fibrosis that affects oral cavity, pharynx and upper third of the oesophagus. The prevalence of the disease is on the rise as a result of increased use of commercially prepared areca nut preparations in India.³

Historical perspective

OSF was first described by Schwartz in 1952⁴ among five Indian females living in Kenya and he coined the term Atrophia idiopathica (trophica) mucosae oris. Several other descriptive terms have been given by subsequent authors, including idiopathic scleroderma of the mouth, idiopathic palatal fibrosis, and sclerosing stomatitis.⁵⁻⁷ Lal highlighted the diffuse nature of OSF⁵ and the condition is no longer considered “idiopathic”.

Our understanding of oral submucous fibrosis has evolved as a clinico-pathological entity over many decades. **Jens Pindborg** - a Danish pathologist - in his various and extensive travels as a WHO consultant to south and far east of globe in search of tropical diseases, comprehensively described various facets of the condition. Pindborg with several Indian colleagues initiated an extensive study on the natural history of oral precancer in a rural population of India. Their journey began at Tata Institute of Fundamental Research (TIFR) in Mumbai with several field stations spread across India. At the time OSF was an enigma to both Jens and to the TIFR team. Field studies planned by Jens and undertaken by the TIFR team led to a breathtaking study that comprised of examining over 200,000 Indian villagers over a span of 30 years. A great clinician and a scientist, his sincerity to work in the villages of India with no basic amenities available, showed the man's motivation and caliber of commitment. Pindborg's art of observations and determined follow up of his observations on this condition over 2 decades lead to several logical conclusions including the premalignant nature of OSF. Supported by the statistical parlance of Prakash Gupta the team led by Fali S. Mehta and Jens Pindborg published their findings in over 30 scientific papers, describing the epidemiology and clinicopathologic aspects of OSF, as we know of today⁸⁻¹⁵.

Aetiology

Until about a decade and half ago the aetiology of the disease was thought to be multifactorial and several agents have been reported, including local irritants (chilies), nutritional deficiency,

and auto-immune disease. However, more recent studies have confirmed areca nut as the major (and the only) risk factor of oral submucous fibrosis, among people who probably have a genetic predisposition to the disease. Sufficient evidence on its etiological role based on epidemiological, animal and *in vitro* studies has been assembled by the International Agency for Research on Cancer and is elegantly presented in several of their monographs¹⁶⁻¹⁸. Areca nut (Fig 1) may be consumed alone or as an ingredient of betel quid. The terminology of various areca consumption habits described by Gupta and Warnaklasuriya¹⁹ and IARC (2004)¹⁷ is listed in Table 1. The role of other ingredients in betel quid (leaf, slaked lime or tobacco) as causative of OSF has not been established.¹⁷ In several cross sectional studies from Taiwan where betel quid is used without added tobacco, significant association of areca/ betel quid use with OSF were found.¹⁸

In 1995, Murti *et al* published a review on the aetiology of OSF²⁰ and the topic was discussed during an expert symposium in London²¹. At the time reported ecological observations pointed towards a possible hypothesis of an association of areca nut with OSF, but the data was weak for inferring a causal relationship. Over the past 15 years additional data have emerged crystallising our understating on the aetiological role of areca nut in causing OSF. However, only an estimated 1-2% of the population who have an areca nut chewing habit may develop the disease. This suggests a possible genetic predisposition in the affected people (see later).

In this review we update the current evidence from primary studies that lead to the IARC's conclusions. Eligible studies were identified from the Monographs published by IARC^{17,18} and more recent literature. A narrative review is present here.

Epidemiological studies

OSF is predominantly encountered in people of South and South East Asia (Fig 2) or among the diaspora arising from these countries. The evidence on the role of areca nut use increasing the risk for development of OSF is based on case reports, case-series studies, prospective cohort studies, and several case-control studies conducted in India, Pakistan Sri Lanka and Taiwan. The relative risk estimates for OSF reported in case-control studies from these 4 countries ranged from 1.8-172 (Table 2). Large confidence intervals noted in many studies are due to "small cell

sizes” in the 2x2 tables in these studies. A summary outcome of these studies by each country is presented below:

India: Five case-control studies from India conducted in Bhavanagar, Nagpur, Kerala, Patna and Chennai provide the relative risk estimates on the use of areca nut²²⁻²⁶. Sinor *et al* provided the first convincing epidemiological study by comparing chewing habits of mawa and betel quid of 60 OSF cases with 60 controls. The reported relative risks were 109.6 for all forms of areca nut chewing, commonly in the form of mawa, primary ingredient of which was areca nut. Subsequent studies from other parts of India (Dharwad) have confirmed these findings²⁷. Hazarey *et al*²⁸ reported significant differences in chewing habits between men and women (males chewing Gutkha, mawa and kharra and women chewing exclusive areca nut). Men were reported to develop the disease at a younger age.

Pakistan: Only case-control study from Pakistan to evaluate the etiology of OSF was conducted in Karachi by Maher *et al*²⁹. They reported various chewing habits of 157 subjects diagnosed with OSF, and estimated RR by comparison with 157 hospital controls. This study estimated a high relative risk of 154 (95% CI 34-693) for areca nut alone users in this Karachi population.

Sri Lanka: One study conducted in a hospital setting (74 cases and 74 controls) confirmed a strong association of OSF with Betel Quid (BQ) chewing (OR = 171.83, 95% CI: 36.35–812.25). In Sri Lanka, areca nut is the primary ingredient of BQ.³⁰ The numbers chewing areca nut alone were meager, and the study did not find any significant risk.

Taiwan: The significance of Taiwanese studies that add weight to areca nut as causative relates to the use of betel quid without any added tobacco by this population. Three forms of betel quid are used in Taiwan, all with areca nut as the main ingredient: betel quid, lao-hwa quid and stem quid.³¹ All reported studies have indicated a significant association of OSF with areca nut or betel quid use.³¹⁻³⁶

The percentage of subjects with an areca nut habit was close to 100% among OSF cases in these observational studies. Case reports that describe fibrosis in non chewers, probably had falsified habit histories or fibrosis arising from other inflammatory disorders .

There are also case-series reports among Indian migrants living in other countries particularly South Africa and the UK which indicates continuation of their areca nut chewing habit following migration^{37,38} Few cases are however, reported among citizens of non-Indian origin.^{39,40}

Dose response

In toxicological studies it is necessary to demonstrate a dose response to confirm a causal effect of an agent under study. In the case of areca nut and betel quid several studies have demonstrated a dose response by examining the frequency and the duration of use. Most studies conducted so far show an increased relative risk with longer duration of use and higher daily consumption (Table 3). There is also clear evidence indicating that with an upsurge of manufactured products containing areca nut (pan masala and Gutka – see Table 1) arriving in markets in India the disease prevalence has increased significantly³ and OSF is being diagnosed at younger ages.⁴¹ This could be due to a rapid development of the disease or starting the chewing habit at a very early age.

Experimental animal studies

Few studies have reported *in-vivo* data on the ability of the areca nut extracts to produce OSF in animal models., Huang, Ling and Wu⁴³ described a rat model of OSF in Hunan Medical University, China and earlier *in-vivo* experiments of Khrime et al⁴⁴ showed histopathological findings akin to OSF induced by *pan masala* on the rat mucosa. The characterizations of these models were not complete and the experimental evidence was neither convincing nor reproducible. The relevance of a particular animal model to a human disease rests on its ability to parallel the biological changes that characterize the disease in humans

Perera et al⁴⁵ described an OSF animal model in female albino mice of BALB/c strain. They applied an aqueous areca nut extract prepared from fresh, mature endosperms of *Areca catechu*

by dissolving nuts in 0.9% normal saline (50 mM NaCl) on the buccal mucosae of mice (n=40) for 600 days. Their study showed fibrosis of treated buccal mucosa as a continuous process occurring in the subepithelial buccal mucosal tissues of treated mice. (Fig 3) The amorphous areas confirmed by van Gieson and Masson's trichrome stains, was an indication of early hyalinization, reflected the presence of young collagen or altered ground substance or both. The findings confirming the excessive deposition of collagen in the treated animals reported by them did bear a close similarity to human OSF.

In this *in-vivo* mouse model the effects of areca nut extract on epithelial thickness leading to atrophy, connective tissue fibrosis, progressive reduction of fibroblasts, and inflammatory changes, were closely similar to that found in human OSF⁴⁶. The experimental data presented by Perera *et al*, further supports areca nut contributing to the causation of OSF.

In vitro studies

Several investigators have studied the effects of constituents of areca nut, such as arecolene and arecaidine on oral fibroblasts *in vitro* in order to provide corroboratory evidence of its cause and effect. The addition of arecolene and arecaidine has shown stimulatory effects on fibroblasts in culture⁴⁶⁻⁴⁸. In a later study, fibroblasts when subjected to different concentrations of aqueous concentrations of raw or boiled areca nut showed morphological alterations⁴⁹. In other *in vitro* studies fibroblasts from OSF specimens showed a more than a 1.5 fold increase in production of collagen compared with fibroblasts from age and sex matched and passage-matched normal controls⁵⁰.

Summary on aetiology

A comprehensive evaluation of data reported from epidemiological, *in vivo* and *in vitro* studies led the IARC (2004)¹⁷ to confirm the etiological role of areca nut as the causative agent of OSF. It is extremely uncommon to find signs of OSF in a non areca nut chewer. Few case reports that describe OSF in never users appear to be misclassification of the disorder due to finding of sclerotic fibrous bands rarely encountered in other chronic inflammatory disorders (eg ulcerative lichen planus or o-GVHD).

Aetiopathogenesis

Although the disease was described in 1950s, its pathogenesis has not been clear until recently. Three previous reviews⁵¹⁻⁵³ had undertaken to critically examine the scientific data available on the pathogenesis of OSF published till 2013. This review updates these research findings. Several mechanisms and biological pathways have been proposed for the pathogenesis of the disorder, all based on the constituents of areca nut and genetic susceptibility to the disease. The flow chart shown below illustrates the possible biochemical and molecular events known in the pathogenesis of OSF (Figure).

Mechanisms of pathogenesis of oral submucous fibrosis

Changes in the extracellular matrix

Areca nut alkaloid, arecoline is now identified as the principal causative factor for OSF. Arecoline appears to be involved in the pathogenesis of OSF causing fibroblastic proliferation and increased collagen formation.

Involvement of connective tissue growth factor (CTGF) in fibrosis in many human tissues is well established. CTGF is only produced by hepatic stellate and kidney mesangial cells in adults under normal conditions, Deng *et al*⁵⁴ have shown expression of CTGF in OSF fibroblasts and endothelial cells in all the OSF cases included in their study. Their in vitro data have shown that arecoline stimulated CTGF production in buccal mucosal fibroblasts through generation of reactive oxygen species (ROS), and by the activation of NF – kappa B, JNK and p38 MAPK pathways. It is also known that NF- kappa B, JNK and p38 are strongly activated by ROS. Significance of ROS in the pathogenesis and malignant transformation of OSF.⁵⁵ has been highlighted.⁵⁵

Arecoline influences deposition of extra cellular matrix (ECM) by increasing the production of TIMP-1 and the effect is enhanced when fibroblasts are co cultured with keratinocytes.⁵⁶ This data suggested that an interaction of oral keratinocytes and fibroblasts may play an important role in the pathogenesis of OSF.⁵⁶ Interaction of arecoline and keratinocytes may induce the differentiation of myofibroblasts from fibroblasts.⁵⁷ Another study proposed that areca alkaloids induce buccal mucosal fibroblast contraction and persistent fibroblast contraction may induce fibrotic process in OSF.⁵⁸ The fact that arecoline influences ECM was further supported by the evidence for Transglutaminase-2 (TGM – 2) over expression in OSF and its regulation by arecoline. TGM – 2 stabilizes ECM protein by cross linking and has been implicated in several fibrotic disorders.⁵⁹

Reduced vascularity is another hallmark of OSF. In order to highlight this aspect, effects of arecoline on endothelial cells have been investigated. It is reported that at concentrations of 0.4 and 0.8 mM, arecoline induces cytotoxicity and also G₂/M cell cycle arrest and increase sub - G₀ / G₁ population, a hall mark of apoptosis. Further it is observed that prolonged exposure to arecoline (0.1mM) significantly suppress endothelial cell proliferation. Also the exposure to >0.2 mM arecoline decreases the proportion of endothelial cells residing in S phase but increases the cells arresting in G₂/M phase. These findings suggest that the anti-proliferative and cytotoxic effects of arecoline are possibly associated with the alteration of specific cell cycle regulatory proteins. Therefore, it is reasonable to suggest that the endothelial damage leading to impairment of vascular function contribute to the pathogenesis of OSF⁶⁰ These findings explain the decreased vascularity that is observed in histological sections, especially with the progression the disease. Loss of vascularity may lead to atrophy of the epithelium and the resulting hypoxic environment may predispose the tissue to carcinogenesis.

In addition to arecoline, arecanut contains more active components including other alkaloids (arecaidine, guvacine, guvacoline and arecolinidine), polyphenols (catechin, flavanoids, flavan-3:4-diols, leucocyanidins, hexahydroxyflavans and tannins) and trace elements (sodium, magnesium, chlorine calcium, vanadium, manganese, copper and bromine). Polyphenols of arecanut such as flavanoid, catechin and tannins cause collagen fibers to crosslink, and thereby make them less susceptible to collagenase degradation. The resulting decrease in collagen breakdown in turn leads to increased fibrosis which is the mainstay of the pathogenesis of OSF.⁶¹

Matrix metalloproteinases and Tissue inhibitors of matrix metalloproteinases (MMPs and TIMPs)

Accurate and balanced collagen metabolism is essential to maintain the normal integrity of connective tissue. Equilibrium between two enzyme groups, MMPs and TIMPs is mandatory to achieve the above. In OSF the equilibrium between MMPs and TIMP is disturbed in such a manner that it ultimately results in increased deposition of ECM.. We demonstrated an attenuated expression of MMP1 in OSF tissues compared with normal oral mucosa.⁶² Since MMP-1, is the main human enzyme that degrades fibrillar collagen, its downregulation may lead to a reduction in collagen degradation. In addition, stronger intensity of TIMP-1 in fibroblasts of OSF than in normal oral mucosa suggested improper regulation of proteolytic equilibrium as one of the main factors responsible for the excessive fibrosis in OSF.⁶² The fibroblasts in OSF have a reduced replicative life span as they accumulate senescent cells during the progression of the disease. This is due to the increased amount of ROS and DNA double strand breaks (DDBs)

produced intrinsically by damaged mitochondria. TIMP -1 & 2 are increased in fibroblast cultures of OSF relative to normal and non-diseased pan user controls. Following the introduction of unreparable DDBs into normal cultured oral fibroblasts TIMP-1 and TIMP-2 secretion increased within 5 days. , Furthermore, TIMP-1 production is enhanced when fibroblasts are co cultured with keratinocytes pretreated by arecoline.^{63,64} This highlights an interaction of oral keratinocytes and fibroblasts in the pathogenesis of OSF. Endogenous collagenase activity in OSF tissues is shown to be 3-5 folds less than that in normal oral mucosa, which may also be responsible for collagen accumulation.⁶⁴ Our data have been reproduced in a recent study that has revealed a decrease in intensity of MMP-1 expression in the epithelium and connective tissue of buccal mucosal tissue of OSF compared with normal tissue.⁶⁵

The above studies highlight the evidence for imbalance between MMPs and TIMPs via different mechanisms leading to accumulation of collagen in OSF. Further, the interaction between keratinocytes and fibroblasts is important in pathogenesis suggesting that OSF is an epithelium driven connective tissue disease..

Copper and related structural changes of collagen

The role of copper in the pathogenesis of OSF has been raised as a result of the discovery of a high copper content in arecanut.⁶⁶ Copper dependant enzyme lysyl oxidase is critical for collagen cross linking and organization of ECM.⁶⁷ Salivary copper is found to be higher in arecanut chewers. This finding indicates that soluble copper found in arecanut is released into the oral environment and its buccal absorption may contribute to fibrosis of oral tissues where copper is deposited. This observation suggested a possible local effect of copper in the aetiopathogenesis of OSF.⁶⁸ Salivary copper levels appear to vary from mild OSF to severe cases.⁶⁹ Serum copper levels in OSF is also raised suggesting a systemic effect and increase in serum copper level has

been shown with the advancement of the clinical stage.^{70,71} However, the effect of copper appears to be local in the context of OSF as there is no evidence to suggest that these patients demonstrate any clinical evidence of systemic or organ fibrosis.

Morphological features of ECM Remodelling in OSF

Histopathological evidence shows ECM remodeling with the progression of the disease. Stage-specific alterations in ECM have been reported.⁷² In the early stages of OSF over expression of tenascin, perlecan, fibronectin and collagen type III may be found in the lamina propria and submucosa while extensive and irregular deposits of elastin were found around muscle fibres in the intermediate stage, together with the above molecules. In the advanced stage collagen type I appears to dominate the ECM. Their gene expression levels were varied with the progression of fibrosis. This pattern of ECM remodeling steps in OSF is similar to normal granulation tissue formation and maturation process. Difficulty in opening the mouth may be related to loss of various ECM molecules such as elastin and replacement of muscle by collagen type I.⁷²

Heat shock protein (HSP) 47, a known collagen specific molecular chaperone involved in the processing and/or secretion of procollagen is significantly upregulated in OSF. Treating fibroblast with arecoline was found to elevate HSP 47 m-RNA expression in a dose dependent manner through MEK, PI3K and COX-2 signal transduction pathways.⁷³ Cystatin C, a non glycosylated basic protein is increased in a variety of fibrotic diseases. Cystatin C was found to be upregulated both at m – RNA and protein levels in OSF and arecoline is responsible for this enhancement in a dose dependent manner.⁷⁴ Malondialdehyde (MDA) is a lipid peroxidation end product with the potential to stimulate fibroblasts and to increase collagen production. MDA is significantly elevated as the grading of OSF progressed.⁷⁵

Inflammatory cytokines and Growth factors

Upregulation of various cytokines namely, TGF β 1, TGF β 1p, THBS1, SPP1, TIG1, TGM2 and CTGF and down regulation of BMP7 which is a known negative modulator of fibrosis has been reported.⁷⁶ TGF β is implicated as one of the main triggers for the increased collagen production and decreased matrix degradation pathways in OSF.^{52,77} It has been shown that the expression of TGF- β 1 was significantly upregulated within the connective tissue of OSF compared to normal mucosa.⁶² Treatment of cells with arecanut water extract consisting of polyphenols and alkaloids, has shown that 64% of the differentially regulated genes found in test samples matches with the TGF β induced gene expression profile.⁷⁸ This suggests that arecoline induces TGF β in keratinocytes and furthermore this is observed only in keratinocytes but not in human gingival fibroblast cells, indicating that keratinocytes could be the source of TGF β in OSF. Further, studies have revealed that keratinocytes secrete TGF β through α v β 6 integrin expression.⁷⁹ Same authors have illustrated loss of TGF β expression using the drug tropicamide that blocks α v β 6 integrin confirming that OSF is an epithelial driven connective tissue disease.⁷⁹ Phosphorylation of SMAD -2 was observed following treatment of keratinocytes by catechin, tannin and alkaloids, hence the authors state that arecanut mediated activation of p-SMAD - 2 involves upregulation and activation of TGF β .⁷⁸ CTGF/CCN2 and COX-2 were found to be over expressed in OSF. The CCN2 synthesis in buccal mucosal fibroblasts is stimulated by TGF β 1 and this reaction is mediated via ALK5, JNK and p38 MAPK pathways. Epigallocatechin-3-gallate (EGCG) in turn can completely block TGF β 1 induced CCN2 synthesis by suppressing JNK and p38 in buccal mucosal fibroblasts, which may be useful in controlling OSF.⁷⁷ Loss of adipose tissue in OSF was found to be due the ability of TGF β to cause lipodystrophy. Level of TGF β secretion is more during the early course of the disease.⁸⁰ b-FGF is increased in

fibroblasts and in endothelial cells in early disease, and in advanced fibrosis b-FGF expression was noted more in stroma.⁸¹ IGF-1 expression is significantly upregulated in OSF both at mRNA and protein levels and arecoline had been responsible for this elevation in a dose dependant manner.⁸² Recently it was reported that the homozygous wild genotype TNF- α 2 was significantly associated with an increased risk of OSF and the mutant allele TNF- α 2 is about 7 times more efficient in promoter function than the wild allele. Accordingly, TNF-may play a role in pathogenesis of OSF through modulation of collagen metabolism.⁸³ With the available literature on growth factors and cytokines, TGF β appears to be the main mediator of the disease and others such as TNF- α , IGF-1, b-FGF and CTGF may contribute to continuous accumulation of collagen with activation of signaling pathways such as ALK5, JNK, SMAD and p38 MAPK. These molecules and pathways can be the target areas for new treatment strategies.

Epithelial–mesenchymal transition (EMT)

Epithelial–mesenchymal transition (EMT) has gained significant attention due to its implication in cancer and fibrosis. Cell injury caused by areca nut extract produces ROS which in turn triggers both MAPK and NF- κ B pathways involved in EMT of OSF. Thereby exposure to areca nut extract causes alterations of normal keratinocyte morphology and induces the cell cycle arrest at G1/S phase and the senescence-associated phenotypes. Keratinocytes secrete a variety of inflammatory mediators such as PGE2, IL-6, TNF- α and most importantly TGF- β , in response to injury. Hif-1 α enhances the EMT in vitro and promotes fibrogenesis by increasing expression of extracellular matrix–modifying factors and lysyl oxidase genes. Further, it is evident that both ROS and HIF- α were necessary for hypoxia-induced TGF- β 1 upregulation. In

OSF a possible relationship between Hif-1 α , ROS and EMT has been revealed as we have shown an upregulation of Hif-1 α ⁸⁴ in OSF and also production of ROS by arecoline treatment.

In summary the above events show possible EMT in OSF leading to fibrosis. Potentially, the transformed epithelial cell may proliferate to expand the fibroblast population, undergo apoptosis, or revert back to epithelial type. The fact that the transformed cell is able to dedifferentiate back to epithelial form directs future research into a novel path as it hints towards a possibility of reversing the disease process.⁸⁵

Molecules in cell cycle control and OSF

There are few studies performed to find out the effects of cell cycle regulation and pathogenesis of OSF. ROS generated by arecoline may cause cell cycle arrest at the G1/G0 phase in human keratinocyte cells without affecting the expression of p21/Cip1. Further, oxidative stress may induce epithelial cell death without eliciting apoptosis at higher arecoline concentration. However, sub-lethal concentrations of arecoline upregulated the expression of several stress responsive genes namely; heme oxygenase-1, ferritin light chain, glucose-6-phosphate dehydrogenase, glutamate-cysteine ligase catalytic subunit and glutathione reductase.⁸⁶ Similar study reported that 0.01-0.04mM arecoline caused late-S and G2/M phase cell cycle arrest in human KB epithelial cells. A decrease in cdc2 and Cyclin B1 protein levels and increasing p21 were identified in gingival keratinocytes by Western blot analysis. It is also revealed that inhibition of KB epithelial cell growth in a dose and time dependant manner and a reduction in

cell number with higher arecoline concentration. Further increase in arecoline concentration has induced both cell necrosis and apoptosis.⁸⁷

Genetic polymorphisms predisposing to OSF

There are estimated to be over 600 million areca nut chewers worldwide¹⁹. However, only 1-2% of areca nut chewers may develop the disease. This suggests a possible genetic predisposition in the affected people. Rapid development of OSF in young adults or even children reported in clinical case reports⁴² further adds weight to this hypothesis. HLA typing has shown certain HLA antigens with a high significantly raised frequency in OSF patients..

Although the exact mechanisms are not clear, various chromosomal, genetic and molecular alterations are associated with the pathogenesis of OSF.⁸⁸ A study using Oligonucleotide microarray has shown 716 genes upregulated and 149 genes were downregulated in OSF. It is identified that genes are involved in immune response, inflammatory response and EMT induced by TGF β signaling pathway namely SFRP4, THBS1, MMP2, ZO-1.⁸⁹ In another study, differentially expressed genes in OSF had been analyzed using bio informatic tools and the genes were located on chromosome 1,2,5,6,7,11 and 12. Gene ontology (GO) classification identified these genes to be related to cellular component sub groups associated with extra cellular matrix, cytoskeleton and cell membrane and also biological process subgroups associated with protein binding, signal transducer activity, immune and defense responses.⁹⁰

Polymorphisms of various genes may also contribute to an increased susceptibility to OSF. Polymorphism of Cytochrome P450 3A gene family is considered as a major determinant of

inter-individual variability in chemical pharmacokinetics. Cytochrome P450 had been identified as a genetic biomarker for susceptibility to develop OSF. This may be helpful in identifying high risk individuals according to the genetic polymorphisms in some exclusive regions of the Cytochrome P450 3A, P4501A1 and CYP2E1 genes.^{91,92} Another study revealed the fact that CYP1A1 (m1) genotype and (m2) genotype singly acts as a protective factor but in the absence of GSTM1 and/or GSTT1 gene significantly alters risk towards the disease.⁹³ A few genes related to the pathway of CYP metabolism such as CYP2B6, CYP2C18, CYP2F1, CYP3A5, microsomal glutathione S-transferase 2 (MGST2), alcohol dehydrogenase (ADH), UDP glucuronosyl transferase 2B15 (UGT2B15), and ADH1C were found to be down regulated in all stages of OSF, thereby reducing the ability of CYP to metabolize and clear betel nut substances and contributing to the pathogenesis.⁹⁴ Recently, genetic polymorphism of lysyl oxidase, was identified and LOX Arg158Gln appeared to be associated more in elderly OSF patients.⁶⁷ Polymorphisms of collagen-related genes on OSF risk had been investigated. A study focused on the single nucleotide polymorphisms (SNPs) of TGF β -1 gene reported that polymorphism in 5'UTR C-T in TGF beta 1 gene associated with pro-angiogenic pathway has a significant association with OSF.⁹⁵ Association of SNP (-1171 5A->6A) in the MMP-3 promoter region with the 5A alleles has an increased risk for developing the disease⁹⁶ while SNPs in the MMP-2 and MMP-9 promoter region is not associated with susceptibility to OSF.⁹⁷ Relationship between OSF and TNF- α genetic polymorphism (-308), was not confirmed as the genotype distribution of TNF- α (-308) genetic polymorphism show similar distribution among areca chewers and non-areca-chewers.⁸³ N-acetyltransferase 2 locus codes for an enzyme that catalyze acetylation of aromatic amines. Polymorphisms at this specific site can cause improper acetylation of the amines, leading to DNA adduct formation. It is suggested that these polymorphisms can increase

the risk of OSF in men if exposed to arecanut.⁹⁸ It can be summarized that the available evidence support the theory of possible genetic predisposition to the disease.(Table 4)

Conclusion

Oral submucous fibrosis was first described over 50 years ago in Indian migrants to south Africa and in Indian subjects as an idiopathic disorder. Observational studies by Jen Pindborg characterized the potentially malignant nature of this disorder. Oral submucous fibrosis is characterised by the accumulation of excessive ECM in the lamina propria, predominantly type 1 collagen and other collagens. Recruitment and differentiation of fibroblasts from the mesenchymal stem cell compartment is a key event and leads to functioning fibrocytes that lay down collagen. In addition to fibroblasts, myofibroblasts and mast cells may participate in fibrogenesis, tissue repair, and angiogenesis and have been implicated in inducing fibrosis. In this disease areca nut and probably continuous mechanical irritation act as external mediators. Growth factors and cytokines (eg TGF β) are key pro-fibrotic factors. We present here a complex sequence of mechanisms that promote fibrosis of the oral mucosa.

Acknowledgements: We thank Dr Dinesh Daftary (Mumbai, India) for helpful comments on life and works of late Jens Pindborg. Fig 1 is reproduced with kind permission from Editor-in- Chief of Journal of Investigative and Clinical Dentistry.

REFERENCES

1. Warnakulasuriya S. Semi-quantitative clinical description of oral submucous fibrosis. *Ann Dent*. 1987; 46:18-21.
2. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J Oral Pathol Med*. 2007; 36:575-80.
3. Gupta PC, Sinor PN, Bhonsle RB, Pawar VS, Mehta HC. Oral submucous fibrosis in India: a new epidemic? *Natl Med J India*. 1998; 11:113-6.
4. Schwartz J. Atrophia idiopathica (tropica) mucosae oris. Demonstrated at the 11th International Dental Congress, London 1952.

5. Lal D. Diffuse oral submucous fibrosis. *J All India Dent Assoc.* 1953; 26:1-3.
6. Joshi SG. Submucous fibrosis of the palate and the pillars. *Indian J Otolaryngol.* 1953; 4:1-4.
7. Pin SI. Idiopathic scleroderma of the mouth. *Arch Otolaryngol.* 1954; 59:330-2.
8. Pindborg JJ, Chawla TN, Srivastava AN, Gupta D, Mehrotra ML. Clinical aspects of oral submucous fibrosis. *Acta Odontol Scand.* 1964; 22:679-691.
9. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. *Oral Surg Oral Med Oral Pathol.* 1966; 22: 764-79.
10. Pindborg JJ, Mehta FS, Daftary DK. Occurrence of epithelial atypia in 51 Indian villagers with oral submucous fibrosis. *Br J Cancer.* 1970; 24:253-7.
11. Pindborg JJ. Is submucous fibrosis a pre-cancerous condition in the oral cavity? *Int Dent J.* 1972; 22:474-80.
12. Pindborg JJ, Bhonsle RB, Murti PR, Gupta PC, Daftary DK, Mehta FS. Incidence and early forms of oral submucous fibrosis. *Oral Surg Oral Med Oral Pathol.* 1980; 50:40-4.
13. Bhonsle RB, Murti PR, Daftary DK, et al. Regional variations in oral submucous fibrosis in India. *Community Dent Oral Epidemiol.* 1987; 15:225-9.
14. Pindborg JJ. Oral Submucous Fibrosis: A Review. *Annal Acad Med.* 1989; 18:603-7.
15. Murti PR, Gupta PC, Bhonsle RB, Daftary DK, Mehta FS, Pindborg JJ. Effect on the incidence of oral submucous fibrosis of intervention in the areca nut chewing habit. *J Oral Pathol Med.* 1990; 19:99-100.
16. International Agency on Research for Cancer. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 37, Tobacco habits other than smoking: betel-quid and areca nut chewing; and some related nitrosamines. 1985; IARC, Lyon.
17. International Agency on Research for Cancer. IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 85, Tobacco habits other than smoking: betel quid and areca-nut chewing. 2004; IARC, Lyon.
18. International Agency on Research for Cancer. IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 100E, Personal habits and indoor combustion. 2012; IARC, Lyon.

19. Gupta PC, Warnakulasuriya S. Global epidemiology of areca nut usage. *Addict Biol.* 2002; 7:77-83.
20. Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Pindborg JJ, Mehta FS. Etiology of oral submucous fibrosis with special reference to the role of areca nut chewing. *J Oral Pathol Med.* 1995; 24:145-52.
21. Meghji S, Warnakulasuriya S. Oral Submucous fibrosis: an expert symposium. *Oral Dis.* 1997; 3:276-91.
22. Sinor PN, Gupta PC, Murti PR, et al. A case-control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. *J Oral Pathol Med.* 1990; 19:94-8.
23. Hazare VK, Goel RR, Gupta PC. Oral submucous fibrosis, arecanut and pan masala use: a case-control study. *Natl Med J India.* 1998; 11:299.
24. Jacob BJ, Straif K, Thomas G, et al. Betel quid without tobacco as a risk factor for oral precancers. *Oral Oncol.* 2004; 40:697-704.
25. Ranganathan K, Devi UM, Joshua E, Kirankumar K, Saraswathi TR. Oral submucous fibrosis: a case-control study in Chennai, South India. *J Oral Pathol Med.* 2004; 33:274-7.
26. Ahmed MS, Ali SA, Ali AS, Chaubey KK. Epidemiological and etiologocal study of oral submucous fibrosis among gutkha chewers of Patna, Bihar, India. *J Indian Soc Pedod Prev Dent.* 2006; 24:84-9.
27. Bathi RJ, Parveen S, Burde K. The role of gutka chewing in oral submucous fibrosis: a case-control study. *Quintessence Int.* 2009; 40:e19-25.
28. Hazarey VK, Erlewad DM, Mundhe KA, Ughade SN. Oral submucous fibrois: study of 1000 cases from central India. *J Oral Pathol Med.* 2007; 36:12-7.
29. Maher R, Lee AJ, Warnakulasuriya KA, Lewis JA, Johnson NW. Role of areca nut in the causation of oral submucous fibrosis: a case-control study in Pakistan. *J Oral Pathol Med.* 1994;23:65-9.
30. Ariyawardana A, Athukorala AD, Arulanandam A. Effect of betel chewing, tobacco smoking and alcohol consumption on oral submucous fibrosis: a case-control study in Sri Lanka. *J Oral Pathol Med.* 2006; 35:197-201.
31. Yang YH, Lee HY, Tung S, Shieh TY. Epidemiological survey of oral submucous fibrosis and leukoplakia in aborigines of Taiwan. *J Oral Patholl Med.* 2001;30:213-9

32. Yang YH, Lien YC, Ho PS, et al. The effects of chewing areca/betel quid with and without cigarette smoking on oral submucous fibrosis and oral mucosal lesions. *Oral Dis.* 2005; 11;88-94.
33. Lee CH, Ko YC, Huang HL, et al. The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. *Br J Cancer.* 2003; 88;366-72.
34. Chung CH, Yang YH, Wang TY, Shieh TY, Warnakulasuriya S. Oral precancerous disorders associated with areca quid chewing, smoking, and alcohol drinking in southern Taiwan. *J Oral Pathol Med.* 2005; 34:460–6.
35. Chen PC, Pan CC, Kuo C, Lin CP. Risk of oral nonmalignant lesions associated with human papillomavirus infection, betel quid chewing, and cigarette smoking in Taiwan: an integrated molecular and epidemiologic study. *Arch Pathol Lab Med.* 2006; 130:57-61.
36. Yen AM, Chen SC, Chen TH. Dose response relationship of oral habits associated with risk of oral premalignant lesions among men who chew betel quid. *Oral Oncol.* 2007; 43:634-638.
37. Canniff JP, Harvey W, Harris M. Oral sumucous fibrosis: its pathogenesis and management. *Br Dent J.* 1986; 160:12:429-434.
38. Seedat HA, van Wyk CW. Betal-nut chewing and submucous fibrosis in Durban. *S Afr Med J.* 1988; 74:568-71.
39. Laskaris G, Bovopoulou O, Nicolis G. Oral submucous fibrosis in a Greek female. *Br J Oral Surg.* 1981; 19:197-201.
40. Simpson W. Submucous fibrosis. *Br J Oral Surg.* 1969; 6:196-200.
41. Babu S, Bhat RV, Kumar PU, et al. A comparative clinico-pathological study of oral submucous fibrosis in habitual chewers of pan masala and betelquid. *J Toxicol Clin Toxicol.* 1996 ;34;317-22.
42. Hayes PA. Oral Submucous Fibrosis in a 4 year old girl. *Oral Surg Oral Med Oral Pathol.* 1985; 59:475-8.
43. Huang S, Ling T, Wu H. [Experimental study on aqueous areca nut extracts inducing oral submucous fibrosis in rats. I. Observation of histomorphology]. *Hua Xi Kou Qiang Yi Xue Za Zhi.* 1997 ;15:91-3, 96.
44. Khrame RD, Mehra YN, Mann SB, Mehta SK, Chakraborti RN. Effect of instant preparation of betel nut (pan masala) on the oral mucosa of albino rats. *Indian J Med Res.* 1991 ;94:119-24.

45. Sumeth Perera MW, Gunasinghe D, Perera PA, et al. Development of an in vivo mouse model to study oral submucous fibrosis. *J Oral Pathol Med*. 2007; 36:273-80.
46. Canniff JP, Harvey W. The aetiology of oral submucous fibrosis: the stimulation of collagen synthesis by extracts of areca nut. *Int J Oral Surg*. 1981; 10:163-7.
47. Harvey W, Scutt A, Meghji S, Canniff JP. Stimulation of human buccal mucosa fibroblasts in vitro by betel-nut alkaloids. *Arch Oral Biol*. 1986; 31:45-9.
48. Jeng JH, Lan WH, Hahn LJ, Hsieh CC, Kuo MY. Inhibition of the migration, attachment, spreading, growth and collagen synthesis of human gingival fibroblasts by arecoline, a major areca alkaloid, in vitro. *J Oral Pathol Med*. 1996 ;25:371-5.
49. Saraswathi TR, Sheeba T, Nalinkumar S, Ranganathan K. Effect of Glutathione on Area Nut Treated Normal Human Buccal Fibroblast Culture. *Indian J Dent Res*. 2006; 17:104-10.
50. Kuo MY, Chen HM, Hahn LJ, Hsieh CC, Chiang CP. Collagen biosynthesis in human oral submucous fibrosis fibroblast cultures. *J Dent Res*. 1995; 74:1783-8.
51. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. *Oral Oncol*. 2006; 42:561-8.
52. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis--a collagen metabolic disorder. *J Oral Pathol Med*. 2005; 34:321-8.
53. Ekanayaka RP, Tilakaratne WM. Oral submucous fibrosis: review on mechanisms of pathogenesis and malignant transformation. *J Carcinogene Mutagene*. 2013; S5: 002. doi:10.4172/2157-2518.S5-00
54. Deng YT, Chen HM, Cheng SJ, Chiang CP, Kuo MY. Arecoline-stimulated connective tissue growth factor production in human buccal mucosal fibroblasts: Modulation by curcumin. *Oral Oncol*. 2009; 45: e99-e1052.
55. Pitiyage GN, Slijepcevic P, Gabrni A, et al. Senescent mesenchymal cells accumulate in human fibrosis by a telomere – independent mechanism and ameliorate fibrosis through matrix metalloproteinases. *J Pathol*. 2011; 223:604-17.
56. Xia L, Tian-You L, Yi-Jun G, Dong-sheng T, Wen-Hui L. Arecoline and oral keratinocytes may affect the collagen metabolism of fibroblasts. *J Oral Pathol Med*. 2009; 38: 422–426.
57. Li X, Ling TY, Gao YJ. Effect of arecoline on the differentiation of myofibroblasts of oral mucosa. *Zhonghua Kou Qiang Yi Xue Za Zhi*. 2007; 42:423-7.

58. Chang MC, Lin LD, Wu HL, et al. Areca nut- induced buccal mucosa fibroblast contraction and its signaling: a potential role in oral submucous fibrosis-a precancer condition. *Carcinogenesis*. 2013; 34:1096-104.
59. Thangjam GS, Agarwal P, Khan I, et al. Transglutaminase-2 regulation by arecoline in gingival fibroblasts. *J Dent Res*. 2009; 88:170-5.
60. Tseng SK, Chang MC, Su CY, et al. Arecoline induced cell cycle arrest, apoptosis, and cytotoxicity to human endothelial cells. *Clin Oral Investig*. 2012; 16:1267-73.
61. Sharan RN, Mehrotra R, Choudhury Y, Asotra K. Association of betel nut with carcinogenesis: revisit with a clinical perspective. *PLoS One*. 2012; 7:e42759. doi: 10.1371/journal.pone.0042759.
62. Illeperuma RP, Ryu MH, Kim KY, Tilakaratne WM, Kim J. Relationship of fibrosis and the expression of TGF- β 1, MMP-1, and TIMP-1 with epithelial dysplasia in oral submucous fibrosis. *Oral Med Pathol*. 2010; 15: 21-28.
63. Pitiyage GN, Lim KP, Gemenitzidis E, et al. Increased secretion of tissue inhibitors of metalloproteinases 1 and 2 (TIMPs -1 and -2) in fibroblasts are early indicators of oral sub-mucous fibrosis and ageing. *J Oral Pathol Med*. 2012; 41:454-62.
64. Lin HJ, Lin JC. Treatment of oral submucous fibrosis by collagenase: effects on oral opening and eating function. *Oral Dis*. 2007; 13:407-13.
65. Mishra G, Ranganathan K. Matrix metalloproteinase-1 expression in oral submucous fibrosis: an immunohistochemical study. *Indian J Dent Res*. 2010; 21:320-5.
66. Khan S, Chatra L, Prashanth SK, Veena KM, Rao PK. Pathogenesis of oral submucous fibrosis. *J Cancer Res Ther*. 2012; 8:199-203.
67. Shieh TM, Tu HF, Ku TH, Chang SS, Chang KW, Liu CJ. Association between lysyl oxidase polymorphisms and oral submucous fibrosis in older male areca chewers. *J Oral Pathol Med*. 2009; 38:109-13.
68. Raja KB, Hazarey VK, Peters TJ, Warnakulasuriya S. Effect of areca nut on salivary copper concentration in chronic chewers. *Biometals*. 2007; 20:43-7.
69. Ayinampudi BK, Narsimhan M. Salivary copper and zinc levels in oral pre-malignant and malignant lesions. *J Oral Maxillofac Pathol*. 2012; 16:178-82.
70. Khanna SS, Karjodkar FR. Circulating immune complexes and trace elements (Copper, Iron and Selenium) as markers in oral precancer and cancer : a randomised, controlled clinical trial. *Head Face Med*. 2006; 2:33.
71. Tadakamadla J, Kumar S, Mamatha GP. Evaluation of serum copper and iron levels among oral submucous fibrosis patients. *Med Oral Patol Oral Cir Bucal*. 2011; 16:e870-3.
72. Utsunomiya H, Tilakaratne WM, Oshiro K, et al. Extracellular matrix remodeling in oral submucous fibrosis: its stage-specific modes revealed by immunohistochemistry and in situ hybridization. *J Oral Pathol Med*. 2005; 34:498-507.

73. Yang SF, Tsai CH, Chang YC. The upregulation of heat shock protein 47 expression in human buccal fibroblasts stimulated with arecoline. *J Oral Pathol Med*. 2008; 37:206-10.
74. Chung-Hung T, Shun-Fa Y, Yu-Chao C. The upregulation of cystatin C in oral submucous fibrosis. *Oral Oncol*. 2007; 43:680-5.
75. Shetty SR, Babu SG, Kumari S, Rao V, Vijay R, Karikal A. Malondialdehyde Levels in Oral Sub Mucous Fibrosis: A Clinicopathological and Biochemical Study. *N Am J Med Sci*. 2012;4: 125–128.
76. Khan I, Agarwal P, Thangjam GS, Radhesh R, Rao SG, Kondaiah R. Role of TGF- β and BMP7 in the pathogenesis of oral submucous fibrosis. *Growth Factors*. 2011; 29:119-27.
77. Chang JZ, Yang WH, Deng YT, Chen HM, Kuo MY. EGCG blocks TGF β 1-induced CCN2 by suppressing JNK and p38 in buccal fibroblasts. *Clin Oral Investig*. 2013; 17:455-61.
78. Khan I, Kumar N, Pant I, Narra S, Kondaiah P. Activation of TGF- β pathway by areca nut constituents: A possible cause of oral submucous fibrosis. *PLoS One*. 2012; 7:e51806.
79. Moutasim KA, Jenei V, Sapienza, K, et al. Betel-derived alkaloid up-regulates keratinocyte α 6 integrin expression and promotes oral submucous fibrosis. *J Pathol*. 2011; 223: 366-77.
80. Kale AD, Mane DR, Shukla D. Expression of transforming growth factor β and its correlation with lipodystrophy in oral submucous fibrosis: an immunohistochemical study. *Med Oral Patol Oral Cir Bucal*. 2013; 18:e12-8.
81. Bishen KA, Radhakrishnan R, Satyamoorthy K. The role of basic fibroblast growth factor in oral submucous fibrosis pathogenesis. *J Oral Pathol Med*. 2008; 37:402-11.
82. Tsai CH, Yang SF, Chen YJ, Chou MY, Chang YC. The upregulation of insulin-like growth factor-1 in oral submucous fibrosis. *Oral Oncol*. 2005; 41:940-6.
83. Chiu CJ, Chiang CP, Chang ML, et al. Association between genetic polymorphism of tumor necrosis factor- α and risk of oral submucous fibrosis, a pre-cancerous condition of oral cancer. *J Dent Res*. 2001; 80:2055-9.
84. Tilakaratne WM, Iqbal Z, Teh MT, et al. Upregulation of HIF-1 α in malignant transformation of oral submucous fibrosis. *J Oral Pathol Med*. 2008; 37:372-7.
85. Yanjia H, Xinchun J (2007) The role of epithelial-mesenchymal transition in oral squamous cell carcinoma and oral submucous fibrosis. *Clin Chim Acta*. 2007; 383:51-6.
86. Thangjam GS, Kondaiah P. Regulation of oxidative-stress responsive genes by arecoline in human keratinocytes. *J Periodontal Res*. 2009; 44:673-82.

87. Lee PH, Chang MC, Chang WH, et al. Prolonged exposure to arecoline arrested human KB epithelial cell growth: regulatory mechanisms of cell cycle and apoptosis. *Toxicology*. 2006; 220:81-9.
88. Mithani SK, Mydlarz WK, Grumbine FL, Smith IM, Califano JA. Molecular genetics of premalignant oral lesions. *Oral Dis*. 2007; 13:126-33.
89. Hu Y, Jian X, Peng J, Jiang X, Li N, Zhou S. Gene expression profiling of oral submucous fibrosis using oligonucleotide microarray. *Oncol Rep*. 2008; 20:287-94.
90. Hu YJ, Jian XC, Liu BJ, Peng JY. Application of bioinformatics tools in analysis of differentially expressed genes in oral submucosal fibrosis. *Zhonghua Kou Qiang Yi Xue Za Zhi*. 2008; 43:168-71.
91. Li N, Hu Q, Jiang C, et al. Novel genetic biomarkers for susceptibility to oral submucous fibrosis: cytochrome P450 3A. *Med Hypotheses*. 2011; 77:834-6.
92. Chaudhuri SR, Mukherjee S, Paul RR, Halder A, Chaudhuri K. CYP1A1 and CYP2E1 gene polymorphisms may increase susceptibility to Oral Submucous Fibrosis among betel quid chewers of Eastern India. *Gene*. 2013; 513:268-71.
93. Ghosh T, Gupta S, Bajpai P, et al. Association of CYP1A1, GSTM1, and GSTT1 gene polymorphism with risk of oral submucous fibrosis in a section of North Indian population. *Mol Biol Rep*. 2012; 39:9383-9.
94. Xie H, Liu J, Ling TY. Expression of cytochrome P450 related genes in oral submucous fibrosis tissue. *Zhonghua Kou Qiang Yi Xue Za Zhi*. 2012; 47:743-7.
95. Rajendran R, Harish RK, Anil S, Vidyadharan R, Banerjee M. Transforming growth factor- β -1 polymorphisms are infrequent but exist at selected loci in oral submucous fibrosis. *Indian J Dent Res*. 2010; 21:413-9.
96. Chaudhary AK, Singh M, Bharti AC, et al. Synergistic effect of stromelysin-1 (matrix metalloproteinase-3) promoter (-1171 5A->6A) polymorphism in oral submucous fibrosis and head and neck lesions. *BMC Cancer*. 2010; 10:369.
97. Chaudhary AK, Pandya S, Mehrotra R, Singh M, Singh M. Role of functional polymorphism of matrix metalloproteinase-2 (-1306 C/T and -168 G/T) and MMP-9 (-1562 C/T) promoter in oral submucous fibrosis and head and neck squamous cell carcinoma in an Indian population. *Biomarkers*. 2011; 16:577-86.
98. Mukherjee S, Bhowmik AD, Roychoudhury P, Mukhopadhyay K, Ray JG, Chaudhuri K. Association of XRCC1, XRCC3, and NAT2 polymorphisms with the risk of oral submucous fibrosis among eastern Indian population. *J Oral Pathol Med*. 2012; 41:292-302.

Legends to figures

Figure 1. Forms of areca nut used in south Asia, pacific islands and China: (a) Ripe areca fruit (b) unripe areca fruit as consumed in Taiwan (c) areca nut (endosperm of Areca fruit shown in (a) commonly consumed in south Asia (d) areca husk used in mainland China (Reproduced with kind permission of J Inv Clin Dent; Reichart PA, Warnakulasuriya S. Oral lichenoid contact lesions induced by areca nut and betel quid chewing: a mini review. J Investig Clin Dent.. 2012 Aug;3(3):163-6

Figure 2. Global map showing areca nut consuming populations

Figure 3. 300-day areca-treated buccal mucosa in albino mice of BALB/c strain. Markedly atrophic epithelium (green arrow), densely deposited oedematous collagen in the lamina propria, inflammatory exudate (yellow arrow), atrophy of the muscles (white arrow) and less vascular connective tissue are prominent (Reproduced with kind permission of John Wiley & Sons, Inc. J Sumeth Perera MW, Gunasinghe D, Perera PA, et al. Development of an in vivo mouse model to study oral submucous fibrosis. J Oral Pathol Med. 2007; 36:273-80).

)

Figure 4. Schematic diagram showing possible molecules and pathways involved in the pathogenesis of oral submucous fibrosis.

Table 1: GLOSSARY OF ARECA NUT & ITS PRODUCTS

Areca catechu L.: see **areca nut**

Areca fruit: fruit of the palm *Areca catechu* L. — see **areca nut**

Areca nut: nut from the fruit of the *Areca catechu* L. (Palmaeaceae) tree, a palm native to South Asia. The fruit is green when unripe and orange-yellow in colour when ripe and is the size of a small egg. The nut (seed) is separated from the fibrous pericarp and used fresh or dried, or processed by roasting, sun drying, boiling, soaking in water or fermenting. The unripe green areca fruit may also be used. Synonyms include **supari** (in Hindi and other languages in India), *puwak* (Sri Lanka), *gua* (in Sylheti), *mak* (Thailand), *pinang* (Sarawak and Malaysia), *daka* (Papua New Guinea), *pugua* (Guam) and *Kun-ywet* (Myanmar). The term 'areca' is derived by the Portuguese from *Malayalam atrekka* and from the Tamil *aakkay*.

Betel inflorescence: flower of the vine *Piper betle* L.

Betel nut: the term 'betel nut', although commonly used in the scientific literature, has caused considerable confusion and should be avoided. The correct term is **areca nut** because betel vine and areca palm are different plants.

Betel quid: usually prepared by smearing a **betel leaf** with **slaked lime**, to which pieces of **areca nut** are added. **Catechu** may be added. Crushed leaves of cured tobacco and flavouring agents may also be added. The ingredients are folded in the betel leaf and chewed. Known as **paan** in Hindi and other languages in India and **buyo** in the Philippines.

Betel quid may be prepared differently in different parts of the world.

Gutka: commercial preparation of **areca nut** and powdered tobacco, **slaked lime**, **catechu** and other ingredients. Also spelt **gutkha**.

Lao-hwa quid: specific Taiwanese term for unripe areca nut split in half, with inflorescence of *Piper betle* L. inserted in the middle and **slaked lime** added

Mawa: mixture of predominantly **areca nut** pieces with some tobacco and **slaked lime**

Naswar: mixture of powdered tobacco, **slaked lime** and indigo. Popular in Afghanistan and Pakistan. Also spelt **nasswar**, **niswar**

Paan: see **betel quid**. Also spelt **pan**

Pan masala: commercial preparation containing

Pan masala: commercial preparation containing **areca nut**, **slaked lime**, **catechu** and other ingredients, but without tobacco.

Stem quid: specific Taiwanese name for betel quid consisting of unripe **areca nut** split in half, with stem of inflorescence inserted in the middle and **slaked lime** added

Supari: see **areca nut**

Tamol: fermented form of **areca nut**

Zarda: tobacco leaf broken into small pieces and boiled in water with **slaked lime** and spices until evaporation, then dried and coloured with vegetable dyes; usually chewed mixed with **areca nut** and spices

Reproduced from IARC Monograph Vol 85. Lyon, IARC. 2004

ACCEPTED MANUSCRIPT

Authors, study location, publication year, (ref number)	Characteristics of cases	Characteristics of controls	Exposure categories	Relative risk (95% CI)	Adjustment for potential confounders
Sinor et al Gujarat, India 1990 (22)	60 cases	60 controls	Non chewers Areca nut or mawa	1 109.6	
Maher et al Karachi, 1994 (29)	157 cases Attending outpatient clinic	157 controls Attending outpatient clinic for other reasons	Non chewer Pan Pan + TOBACCO Areca nut	1 32 (6-177) 64 (15-274) 154 (34-693)	
Yang et al (2001) (31)	312 participants (119) men, 193 women)	out of a source population of 3623 in Mutan Country (aboriginal Community)	Non chewer Areca/BQ chewer	1.0 1.8 (0.7-4.8)	Adjusted for each other, age and gender
Lee et al (2003) Kaohsiung 1994-95 (33)	125 histologically confirmed cases of OSF (93 men, 1 woman)	876 population controls (844 men, 32 women) matched on age and sex	Never chewed Former chewer Current chewer	1 12.1 (2.8-51.9) 40.7 (16.0 -103.7)	Adjusted for education and occupation
Jacob <i>et al</i> (2004) Kerala, India (24)	170 Oral submucous fibrosis	47 773 subjects with no oral mucosal disorders from the same screening study	Non chewer Chewer (betel quid only) Chewer (betel quid with tobacco)	1.00 56.2 (21.8-144.8) 73.0 (32.9- 162.2)	
Yang et al (2005) Mutan community, Taiwan (32)	62 subjects Detected by screening	62 controls selected from the same screening programme	Non-chewer Chewer (betel quid only)	1.00 4.51 (1.20-16.94)	Non smoker
Ranganathan et al (2004) Chennai South India (25)	185 cases	185 hospital-based controls	Non chewer Chewer AN Chewer (pan Masala)	1 3.10 (0.83±11.65) 81.50 (4.95-1341.12)	
Chung et al (2005) Taiwan (34)	17 cases	1075 subjects examined	Non-chewer Areca quid	1.00 151.9 (19.1-999)	Included smokers
Ariyawardana et al (2006) Sri Lanka (30)	74 (61 men, 13 women) Hospital-based	74 (61 men, 13 women)	Non-chewer Areca nut Betel quid	1.00 11.79 (0.64–217.2) 16.24 (5.8–44.8)	Non Tobacco or alcohol consumption
Chen et al (2006) Taiwan (26)	23 cases of submucous fibrosis (among 113 pathology archives)	23 control and 27 cases of non-premalignant disorders	Non-chewer Chewer (betel quid only)	1.00 4.2 (0.17-0.54)	
Ahmad et al (2006) Patna, India (26)	157 Oral submucous fibrosis cases hospital based	135 Hospital-based controls with other diseases	Areca nut only Pan masala Pan Gutka	172.8 9 (15.87-723.27) 138.21 (32.97- 629.34) 41.5 (12.33-156.59) 234.9 (67.17-900.330)	
Yen et al (2008) China, Taiwan (36)	441 oral submucous fibrosis,	8360 men Participating in a screening	Occasional use +20 pieces/day	1 6.89 (4.96-9.58)	Age, Education, occupation, smoking & alcohol drinking

Table 1: Epidemiological studies confirming the association of areca / betel quid use with oral sub mucous fibrosis in India, Pakistan, Sri Lanka and Taiwan (Adapted from IARC Monographs 2004, 2012). Large confidence intervals noted in many studies are due to “small cell sizes” in the 2x2 tables in these studies.

Author, year, reference number	Quids/day	Odds ratio (95% CI)	Duration of chewing (years)	Odds ratio (95% CI)
Maher et al (1994) (29)	0 1-5 6-10 10+	1 84 (20-360) 246 (47-1278) 100 (19-522)	0 1-5 6-10 10+	1 72 (17-316) 137 (29-640) 109 25-479)
Yang et al (2001) (31)	1-10 11-20 >21	1.0 1.2 (0.7-2.04) 1.3 (0.7-2.2)	1-10 11-20 21-30 >31	1 1.8 (0.7-4.8) 2.4 (1.0-5.0) 2.4 (1.1- 5.0)
Lee et al (2003) (33)	1-10 11-20 >21	31.4 (11.9-82.5) 37.4 (12.6-110.4) 53.5 (16.4-174.8)	1-10 11-20 >21	30.9 (11.3-84.7) 41.9 (14.1-124.9) 39.3 (11.7-131.7)
Yang et al (2005) (32)	1-9 10-29 30+	3.66 (0.71-18.91) 4.55 (1.16-17.84) 10.34 (2.30-44.73)		
Yen et al (2008) (36)	Occasional 1-10 11-20 20+	1 1.26 (0.91-1.74) 3.88 (2.75-5.60) 6.98 (4.96-9.58)		

Table 3: Dose response relationship of areca habits and OSF

Genetic Polymorphism	Role in pathogenesis of OSF	Reference
Cytochrome P450	A genetic biomarker for susceptibility to OSF	91
Cytochrome P450 3A, P4501A1 , CYP2E1	Marks high risk individuals	92
CYP1A1 (m1) and (m2) genotypes	Confirms protection	93
Lysyl oxidase		
LOX Arg158Gin	Associated more in elderly OSF patients	67
TGFβ -1 (single nucleotide polymorphism in 5'UTR C-T)	Increased risk for developing OSF	95
MMP-3 (single nucleotide polymorphism in -1171 5A->6A)	Increased risk for developing OSF	96
TNF-α (-308)	No confirmed relationship with OSF	83
N-acetyltransferase 2 locus	Increased risk for developing OSF (men only	98

Table 4: Genetic polymorphisms predisposing to OSF or confirming protection





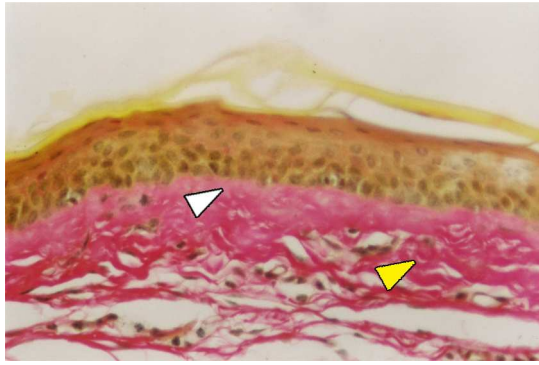
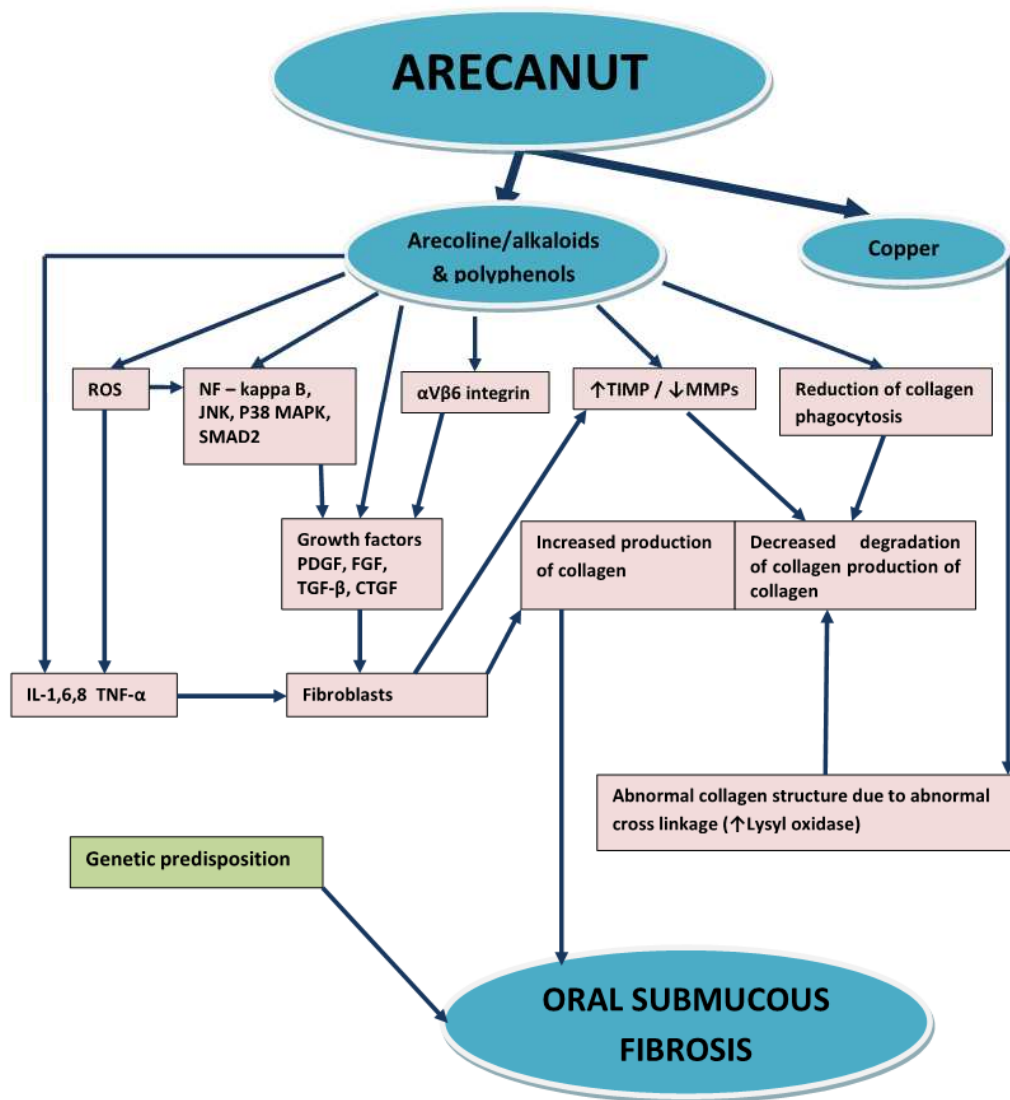


Figure 4: Possible molecules and pathways involved in the pathogenesis of Oral submucous fibrosis.



Statement of clinical relevance

Oral submucous fibrosis remains unfamiliar to clinicians in the Americas and Europe. Knowledge of the aetiology of this disease helps to identify “high risk” individuals and understanding the aetiopathogenesis of OSF may contribute to new research to develop targeted therapies.