Oral Submucous Fibrosis

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8.1 Introduction

Oral submucous fibrosis (OSF) is a chronic, insidious disease characterised by progressive submucosal fibrosis of the oral cavity and the oropharynx. The disease sometimes extends to the pharynx and upper third of the oesophagus. As the disease progresses, the resulting loss of fibroelasticity and stiffening of the oral mucosa leads to limitation in opening of the mouth of affected individuals. The presence of fibrous bands in lips, cheeks and soft palate is a hallmark of the disease [1].

8.2 Historical Perspective

OSF was first described by Schwartz in 1952 [2] among five Indian females living in Kenya, and he coined the term atrophia idiopathica (trophica) mucosae oris. Joshi (1953) [3] coined the term submucous fibrosis based on morphological characteristics of the disease. In the same year, Lal (1953) [4] recognised the widespread

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diffuse nature of the disease affecting the whole oral mucosa. A year later, Pin [5] described a case series from Taiwan (formerly Formorsa) naming the condition idiopathic scleroderma of the mouth. Several other descriptive terms have been given by subsequent authors: idiopathic palatal fibrosis and sclerosing stomatitis. The premalignant nature of the disease was first reported by Paymaster in 1956 [6].

Oral submucous fibrosis has evolved as a clinicopathological entity over many decades, with the current clinical significance being accepted worldwide following its rediscovery by late Jens Pindborg who described the epidemiology and clinicopathologic aspects of OSF [7–12]. The topic was discussed during expert symposia in London [13], in Kuala Lumpur [14] and at the World Workshop on Oral Medicine V in 2010 [15].

8.3 Epidemiology

OSF is exclusively described among populations in India, Pakistan, Sri Lanka, Nepal, Taiwan and among the Pacific Islanders, but sporadic cases have been described from Southern China, South Vietnam, Thailand (Fig. 8.1) and among migrants from the Indian subcontinent to the UK, USA and South and East Africa. Exact prevalence figures of OSF can only be extrapolated from large house-to-house surveys. The prevalence of OSF was found to be 0.36 % in the Ernakulum district in South India [16]; 3.4 % in Durban, South Africa [17]; 3.0 % in Hunan Province, China [18]; and 17.6 % in aboriginal Taiwanese [19]. The lower figure in the Indian survey may be due to strict criteria used by them that banding was necessary to diagnose OSF. Regional variations in the incidence of OSF within in the Indian subcontinent were reported by Bhonsle [20].

In reported screening programmes, 15 cases of OSF were found among 28,295 subjects screened in a field study in Sri Lanka [21] and 23 OSF cases among 10,547 screened in a community programme in Taiwan (Su et al. 2004) [22]. Rising trends

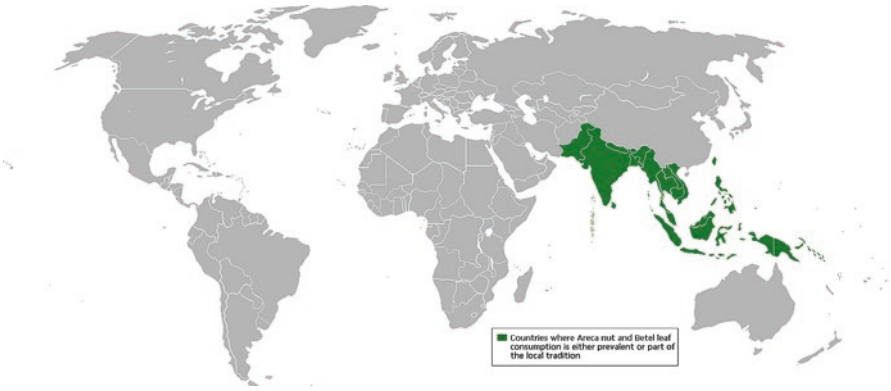

Fig. 8.1 Countries with a high prevalence of areca nut chewing habit (*marked in green*)

are reported in Gujarat, India; a prevalence of 0.16 % reported in 1967 had risen to 3.3 %, two decades later [23].

The incidence rates of OSF were reported in India by Gupta et al. [16] with a slightly higher female predilection, 19 per 100,000 in female and 8 per 100,000 in male. In a 6-year follow-up study among (aboriginal) areca/betel quid chewers in Taiwan, a higher incidence of 374.1 per 100,000 person years was reported [24].

8.4 Aetiology

Based on the epidemiological, animal and *in vitro* data from various studies assembled by the International Agency for Research on Cancer (IARC), it has been shown that areca nut is the sole aetiological factor responsible for causation of OSF. Evidence for this association is presented in several of the IARC monographs [25–27]. A plethora of other aetiological factors reported by various authors such as local irritants (chillies), nutritional deficiency and autoimmune disease are no longer considered to be causative. Areca nut (Fig. 8.2) may be consumed alone or as an ingredient of betel quid, but the role of other ingredients in betel quid (leaf, slaked lime or tobacco)[28] are not considered to be causative

Fig. 8.2 (**a**) Ripe areca fruit (**b**) Unripe areca fruit used in Taiwan (**c**) The endosperm of areca fruit, areca nut (**d**) Dried areca husks used in Souther

factors for OSF. The evidence highlighting the causative role of areca nut has been reviewed by Murti et al. [29] and Tilakaratne et al. [1, 30]. In this chapter we update the current evidence from primary studies that lead to the IARC's conclusions.

8.5 Epidemiological Studies

The evidence on the role of areca nut use in 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. A summary outcome of reported case–control studies conducted among several populations is presented in Table 8.1.

The first reported case control study on OSF was reported from Bhavnagar in India by Sinor et al. [31] by comparing chewing habits of mawa and bêtel quid of 60 OSF cases with 60 controls. The reported relative risks were 109.6 for all forms of areca nut chewing. Later, four other studies from India conducted in Nagpur, Kerala, Patna and Chennai [32–35], one study each from Pakistan [36] and Sri Lanka [37] and six studies from Taiwan [19, 38–42] have indicated a significant association of OSF with areca nut chewing or betel quid use. Two other studies from India described an association without showing statistical evidence [43, 44].

There are also case series reports among Indian migrants living in other countries particularly South Africa and the UK which indicate that the prevalence and frequency of areca nut use among OSF cases is much higher than in the general population [45, 46]. The percentage of subjects with an areca nut habit reported among OSF cases was close to 100 % in these studies. Case reports that describe fibrosis in non-chewers, probably had falsified habit histories [47] or included cases of oral mucosal fibrosis arising from other inflammatory disorders [48, 49].

8.6 Dose Response

A dose response confirms 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 its use. Most studies conducted so far show an increased relative risk with longer duration of use and higher daily consumption (Table 8.2). There is also clear evidence indicating that with an upsurge of manufactured products containing areca nut (pan masala and gutka) arriving in markets in India, the disease prevalence has increased significantly [23], and OSF is being diagnosed earlier (i.e. disease developing rapidly) and at younger ages [50].

Table 8.1 Epidemiological studies confirming the association of areca/betel quid use with oral submucous fibrosis in India [31–35], Pakistan [36], Sri Lanka [37] and Taiwan [19, 38–42]

Authors, study location and publication year	Characteristics of cases	Characteristics of controls	Exposure categories	Relative risk (95 % CI)	Adjustment for potential confounders
Sinor et al. Gujarat, India 1990 [31]	60 cases	60 controls	Non-chewers	1	
			Areca nut or mawa	109.6	
Maher et al. Karachi, Pakistan 1994 [36]	157 cases Attending outpatient clinic	157 controls Attending outpatient clinic for other reasons	Non-chewer	1	
			Pan	32 (6–177)	
			Pan+TOBACCO	64 (15–274)	
			Areca nut	154 (34–693)	
Hazare et al. Nagpur, India 1998 [32]	200 cases attending Dental hospital	197 age-matched controls	Non-chewer	1	
			Areca user	49.3	
Yang et al. (2001) Pingtung 1997 [38]	312 participants (119 men, 193 women)	Out of a source population of 3623 in Mutan Country (aboriginal community)	Non-chewer	1.0	Adjusted for each other, age and gender
			Areca/BQ chewer	1.8 (0.7–4.8)	
Lee et al. (2003) Kaohsiung 1994–95 [39]	125 histologically confirmed cases of OSF (93 men, 1 woman)	876 population controls (844 men, 32 women) matched on age and sex	Never chewed	1	Adjusted for education and occupation
			Former chewer	12.1 (2.8–51.9)	
			Current chewer	40.7 (16.0–103.7)	
Jacob et al. (2004) Kerala, India [33]	170 oral submucous fibrosis	47,773 subjects with no oral mucosal disorders from the same screening study	Non-chewer	1.00	
			Chewer (betel quid only)	56.2 (21.8–144.8)	
			Chewer (betel quid with tobacco)	73.0 (32.9–162.2)	
Yang et al. (2005) Mutan community, Taiwan [19]	62 subjects Detected by screening	62 controls selected from the same screening programme	Non-chewer	1.00	Non-smoker
			Chewer (betel quid only)	4.51 (1.20–16.94)	

(continued)

Table 8.1 (continued)

Authors, study location and publication year	Characteristics of cases	Characteristics of controls	Exposure categories	Relative risk (95 % CI)	Adjustment for potential confounders
Ranganathan et al. (2004) Chennai South India [34]	185 cases	185 hospital-based controls	Non-chewer	1	
			Chewer AN	3.10 (0.83 ± 11.65)	
			Chewer (pan masala)	81.50 (4.95–1341.12)	
Chung et al. (2005) Taiwan [40]	17 cases	1075 subjects examined	Non-chewer	1.00	Included smokers
			Areca quid	151.9 (19.1–999)	
Ariyawardana et al. (2006) Sri Lanka [37]	74 (61 men, 13 women) Hospital-based	74 (61 men, 13 women)	Non-chewer	1.00	Non-tobacco or alcohol consumption
			Areca nut	11.79 (0.64–217.2)	
			Betel quid	16.24 (5.8–44.8)	
Chen et al. (2006) Taiwan [41]	23 cases of submucous fibrosis (among 113 pathology archives)	23 control and 27 cases of non-premalignant disorders	Non-chewer	1.00	
			Chewer (betel quid only)	4.2 (0.17–0.54)	
Ahmad et al. (2006) Patna, India [35]	157 oral submucous fibrosis cases, hospital based	135 hospital-based controls with other diseases	Areca nut only	172.8 9(15.87–723.27)	
			Pan masala	138.21 (32.97–629.34)	
			Paan	41.5 (12.33–156.59)	
			Gutka	234.9 (67.17–900.330)	
Yen et al. (2008) China, Taiwan [42]	441 oral submucous fibrosis	8360 men Participating in a screening	Occasional use	1	Age, education, occupation, smoking and alcohol drinking
			+20 pieces/day	6.89 (4.96–9.58)	

Adapted from IARC Monographs 2004, 2012, Modified from Tilakaratne et al. [1]

Table 8.2 Dose–response relationship of areca habits and OSF

	Quids/day	Odds ratio (95 % CI)	Duration of chewing (years)	Odds ratio (95 % CI)
Maher et al. (1994) [36]	0	1	0	1
	1–5	84 (20–360)	1–5	72 (17–316)
	6–10	246 (47–1278)	6–10	137 (29–640)
	10+	100 (19–522)	10+	109 (25–479)
Yang et al. (2001) [19]	1–10	1.0	1–10	1
	11–20	1.2 (0.7–2.04)	11–20	1.8 (0.7–4.8)
	>21	1.3 (0.7–2.2)	21–30	2.4 (1.0–5.0)
			>31	2.4 (1.1–5.0)
Lee et al. (2003) [39]	1–10	31.4 (11.9–82.5)	1–10	30.9 (11.3–84.7)
	11,020	37.4 (12.6–110.4)	11–20	41.9 (14.1–124.9)
	>21	53.5 (16.4–174.8)	>21	39.3 (11.7–131.7)
Yang et al. (2005) [38]	1–9	3.66 (0.71–18.91)		
	10–29	4.55 (1.16–17.84)		
	30+	10.34 (2.30–44.73)		
Yen et al. (2008) [42]	Occ	1		
	1–10	1.26 (0.91–1.74)		
	11–20	3.88 (2.75–5.60)		
	20+	6.98 (4.96–9.58)		

8.7 Genetic Predisposition

There are over 600 million areca nut chewers reported worldwide [27]. However, only 1–2 % of the population 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 reports [51] further adds weight to this hypothesis. Genetic polymorphisms discovered in affected individuals that may predispose them to the disease are discussed in a later section in this chapter.

8.8 Experimental Animal Studies

In vivo experimental data on the ability of the areca nut extract to produce OSF is meagre. However, Huang, Ling and Wu [52] claim to have produced a rat model of OSF in Hunan Medical University, China, and earlier in vivo experiments of Khrime et al. [53] 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

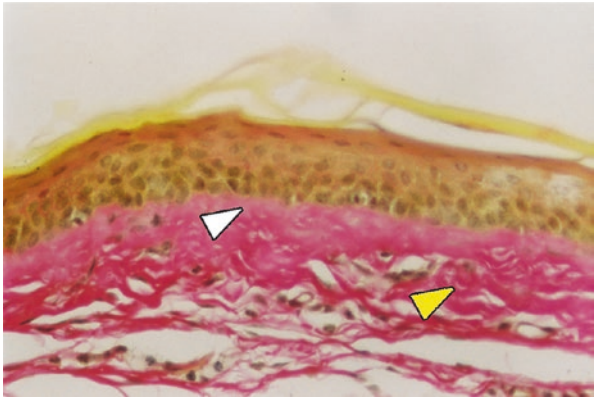

Fig. 8.3 Histopathological features of OSF, illustrating thin atrophic epithelium and fibrosis of underlying connective tissue. *Arrows* show collagen deposition

reproducible. The relevance of a particular animal model to a human disease rests on its ability to parallel the biological changes that characterise the disease in humans.

Perera et al. [54] 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. 8.3). The amorphous areas confirmed by van Gieson and Masson's trichrome stains were an indication of early hyalinization and reflected the presence of young collagen or altered ground substance or both. These findings confirming the excessive deposition of collagen in the treated animals 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 OSF [54]. The experimental data presented by Perera et al. further supports areca nut contributing to the causation of OSF.

8.9 *In Vitro* Studies

Several investigators have studied the effects of constituents of areca nut, such as arecoline and arecaidine, on oral fibroblasts *in vitro* in order to provide corroboratory evidence of cause and effect. The addition of arecoline and arecaidine has shown stimulatory effects on fibroblasts in culture [55–57]. In a later study, fibroblasts when subjected to different concentrations of aqueous concentrations of raw or boiled areca nut showed morphological alterations [58]. In other *in vitro* studies, fibroblasts from OSF specimens showed more than a 1.5-fold increase in production

of collagen compared with fibroblasts from age- and sex-matched and passage-matched normal controls [59].

8.10 Summary on Aetiology

A comprehensive evaluation of above data led the IARC [25, 27] to confirm the aetiological role of areca nut as the causative agent of OSF. In our wide experience from field and clinical studies, we have not encountered any single case of OSF in a non-areca nut chewer. Few case reports that describe OSF in white Caucasians [48, 49] appear to be a misclassification of the disorder due to finding of sclerotic fibrous bands rarely encountered in other chronic inflammatory disorders (e.g. ulcerative lichen planus or chronic oGVHD).

8.11 Aetiopathogenesis

Although the disease was described in 1950s, its pathogenesis has not been clear until recently. Three published reviews [30, 60, 61] had undertaken to critically examine the scientific data available on the pathogenesis of OSF published up to 2015. 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 (Fig. 8.4 – modified from WWOM V) [15].

8.12 Mechanisms of Pathogenesis of Oral Submucous Fibrosis

8.12.1 Constituents of Areca Nut and Their Primary Effects

Areca nut contains active components including alkaloids (arecoline, 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). Arecoline was identified as the principal causative factor for OSF by Caniff's group [55, 56] and appears to be involved in the pathogenesis of OSF by causing fibroblastic proliferation and increased collagen formation. It appears that the main pathological change in OSF is the increased accumulation of type 1 collagen within the subepithelial tissues. Polyphenols of areca nut such as flavanoids, catechin and tannins cause collagen fibres to cross-link 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 [30]. In the past decade, various mechanisms leading to submucosal fibrosis have been demonstrated and these are briefly presented.

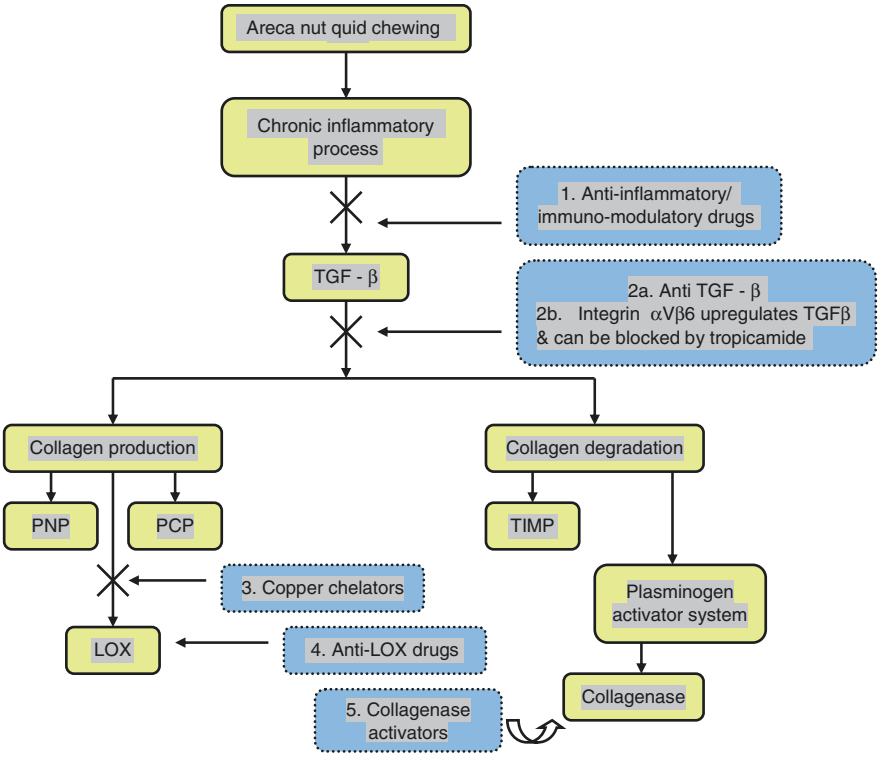

Fig. 8.4 Pathogenesis of oral submucous fibrosis (OSF) – A schematic illustration of the collagen production pathway and potential elements of molecular interventions (Modified from Rajalalitha and Vali [60]). *PCP and PNP* – the enzymes known as the procollagen C and N proteinases (PCP and PNP) are involved in the processing of fibrillar procollagen precursors to mature collagens. *TIMP* – the matrix metalloproteinases are inhibited by specific endogenous tissue inhibitors of metalloproteinases (TIMPs), which comprise a family of four protease inhibitors

8.12.2 Fibrogenic Factors

Several fibrogenic cytokines such as transforming growth factor-β1 (TGF-β), basic fibroblastic growth factor (bFGF) and connective tissue growth factor (CTGF) are associated with fibrosis of different organs. Among these TGF-β is known to be a potent stimulator of extracellular matrix through inducing transdifferentiation of fibroblasts into myofibroblasts [62] (Fig. 8.5). TGF-β has been shown to be expressed in OSF tissues [63–65] (Haque et al. 1998), and the key role of this cytokine in the progression of OSF has been proposed by other authors (Khan et al. 2012) [66]. Several studies have shown that αγβ6-dependent TGF-β1 activation promotes pathogenic organ fibrosis. Moutassim et al. (2011) [67] have demonstrated upregulation of αγβ6 in OSF tissue samples, and their study indicates this as the likely mechanism involved in TGF-β1 activation in OSF by arecoline, leading to

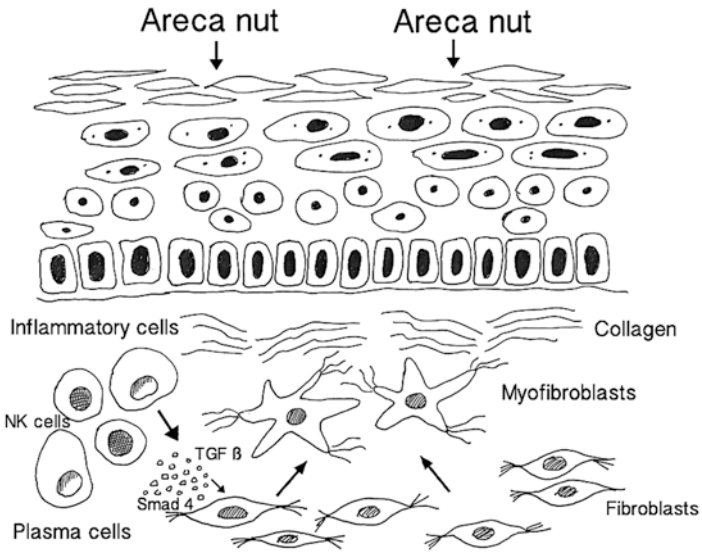

Fig. 8.5 Schematic diagram of fibroblast activation (courtesy of Dr Helen McParland)

fibrosis. Several other growth factors may also be upregulated in OSF such as basic fibroblast growth factor [68] and insulin-like growth factor-1 [69].

Involvement of connective tissue growth factor (CTGF) in fibrosis in many human tissues is well established [70]. Expression of CTGF (ccn2) was reported in fibroblasts in scleroderma patients [71], and a further study has shown that arecoline stimulated CTGF production in buccal mucosal fibroblasts through reactive oxygen species (ROS) [72]. Both CTGF can induce collagen production via TGF-β dependent and independent pathways. However, recent work by Kahn et al. suggests a major causative role for TGF-β that is induced by areca nut in OSF progression [73] (Fig. 8.5).

Experimental studies have shown that mechanical trauma may also induce secretion of TGF-β (Manokawinchoke et al. 2015) [74]. Thus continuous mastication (of areca nut) may have a similar effect and contribute to TGF-β deposition in buccal tissues.

8.12.3 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 extracellular matrix ECM. Immunohistochemical studies have shown that

MMP-1 expression is attenuated in OSF compared to normal oral mucosa [64, 75]. Since MMP-1 is the main human enzyme that degrades fibrillar collagen, this suggests that collagen degradation caused by MMP-1 is reduced in OSF [75]. In addition, stronger intensity of TIMP-1 in fibroblasts of OSF compared to normal oral mucosa suggested improper regulation of proteolytic equilibrium as one of the main factors responsible for the excessive fibrosis in OSF [64]. The fibroblasts in OSF have a reduced replicative life span as they accumulate senescent cells during the progression of the disease [76]. This is due to the increased amount of ROS and DNA double-strand breaks (DDBs) produced intrinsically by damaged mitochondria. TIMP-1 and TIMP-2 are increased in fibroblast cultures of OSF relative to normal and non-diseased paan user controls [77].

8.12.4 Copper and Related Structural Changes of Collagen

The role of copper in the pathogenesis of OSF was raised by the King's College Group as a result of their novel finding of high copper content in areca nut [78]. The copper-dependant enzyme lysyl oxidase is critical for collagen cross-linking and organisation of ECM [79], and this enzyme was found to be upregulated in OSF. Salivary copper is found to be higher in areca nut chewers [80]. Salivary copper levels appear to vary from mild OSF to severe cases [81]. These findings indicate that soluble copper found in areca nut is released into the oral environment and its oral absorption may contribute to fibrosis of buccal mucosa suggesting a possible local effect of copper in OSF patients [82]. Serum copper levels in OSF patients are also raised suggesting a systemic effect, and levels correlate with the advancement of the clinical stage [83, 84]. However, the effects of copper appear to be local in the context of OSF as there is no evidence to suggest that these patients develop systemic fibrosis. Spraying of areca crops with copper sulphide used as a fungicide to preserve the fruit has been attributed as a likely source of high copper in the areca growing belt.

8.12.5 Changes in the Extracellular Matrix

Histopathological evidence shows ECM remodelling with the progression of the disease. It has been reported that in early stage of OSF, tenascin, perlecan, fibronectin and collagen type III are overexpressed in the lamina propria and submucosa [85]. 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. The gene expression levels of these molecules were varied with the progression of fibrosis. This pattern of ECM remodelling steps in OSF is similar to normal granulation tissue formation and maturation process. Difficulty in opening the mouth may be related to the loss of various ECM molecules such as elastin and replacement of muscle by collagen type I [86].

Table 8.3 Genetic polymorphisms predisposing to OSF

Genetic polymorphism	Role in pathogenesis of OSF	References
Cytochrome P450	A genetic biomarker for susceptibility to OSF	[87, 88, 95]
Cytochrome P450 3A, P4501A1, CYP2E1	Helpful in identifying high-risk individuals	
CYP1A1(m1) and (m2)		
Genotypes	Acts as protective factors (in the absence of GSTM1 and/or GSTT1 genes), alters risk towards the disease	[89]
Lysyl oxidase		
LOX Arg158Gin	Associated more in elderly OSF patients	[90]
TGFβ-1 (single nucleotide polymorphism in 5¢UTR C-T)	Associated with pro-angiogenic pathway	[91]
MMP-3 (single nucleotide polymorphism in 1171 5A->6A)	Increased risk for developing OSF	[92, 93]
N-acetyltransferase	Increase the risk of OSF in men	[94]

8.12.6 Genetic Polymorphism Predisposing to OSF

Polymorphisms of various genes have contributed to the pathogenesis of disorders in different ways. Polymorphism of the cytochrome P450 3A gene family is considered as a major determinant of interindividual 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 [87, 88]. The evidence available to support other possible genetic predispositions [89–94] to the disease is summarised in Table 8.3.

8.12.7 Clinical Features

A workshop held in Kuala Lumpur, Malaysia [14], recommended the following clinical criteria for the diagnosis of OSF:

- Presence of palpable fibrous bands
- Leathery mucosal texture
- Blanching of mucosa
- Loss of tongue papillae
- Burning sensation to spicy food
- Rigidity of the tongue

Blanching of the mucosa is an early feature, and some authors refer to this as depigmentation of mucosa [96]. Fibrous banding involving the buccal mucosae (Fig. 8.6), lip or palate (Fig. 8.7) is noted in established stages of OSF. At later stages, this may manifest with a marble-like appearance. Occurrence of small

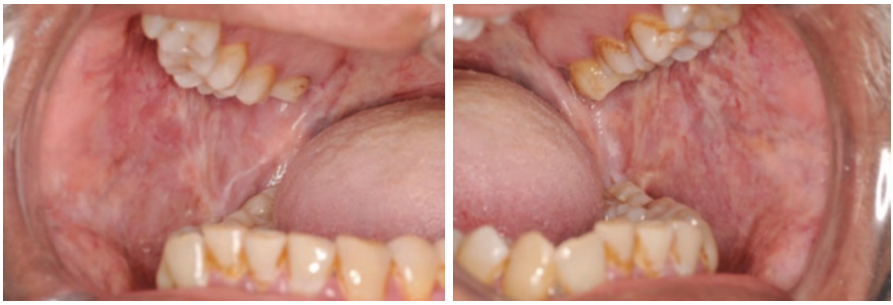

Fig. 8.6 Fibrous bands on buccal mucosa in OSF

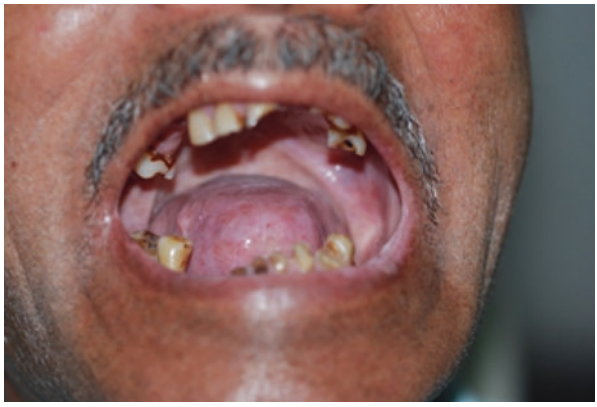

Fig. 8.7 Palatal fibrosis in OSF

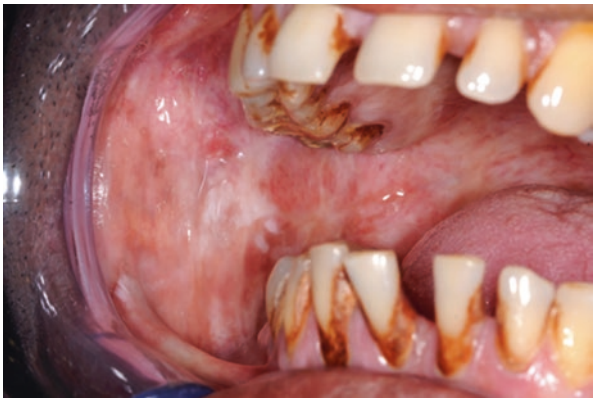

Fig. 8.8 A patch of leukoplakia in a case of OSF

reddish blue spots in a quarter of the patients was highlighted by Bhonsle et al. (1981) [97]. Presence of vesicles has also been reported as an early feature. Due to vertical bands, progressive limitation of mouth opening is a hallmark feature of this disease. Other potentially malignant disorders such as leukoplakia also caused by betel quid may be found to coexist (Fig. 8.8).

Table 8.4 OSF disease grading system proposed by Warnakulasuriya and adapted at WWOM V

Grade 1 – burning, depapillation, blanching or leathery mucosa (disease triad for OSF); mouth opening, >35 mm
Grade 2 – moderate limitation of opening 20–35 mm (+ disease triad and fibrous bands)
Grade 3 – severe OSF, limitation of opening <20 mm
Grade 4A – OSF + other potentially malignant disorder
Grade 4B – OSF with oral epithelial dysplasia
Grade 5 – OSF + SCC

8.12.8 Staging

Several classification schemes have been proposed for staging OSF. These may be based solely on clinical criteria or histopathological features; Warnakulasuriya [98] first reported to use the inter-incisal opening as a semiquantitative clinical measure to assess the worsening of OSF. An opening limited to 35 mm or less was considered as moderate or advanced disease. Maher et al. [99] tested the inter-incisal opening as a measure of severity of OSF and confirmed it correlated well with the extent of the disease found within the oral cavity. Pindborg and Sirsat (1966) proposed a classification based on histopathology [8]. Clinical classifications have been proposed by Pindborg (1989) [12], Lai [100] based on mouth opening to four groups and Ranganathan having first examined normal subjects (2001) and classifying OSF subjects (2006) [101, 102]. Pindborg's 1989 classification did not use mouth opening but staged the disease by mucosal alterations (vesicles, ulceration, pigmentation) and fibrosis. Later authors have used mouth opening as an important factor to grade the severity of the disease. The latest staging proposed at World Workshop on Oral Medicine V [15] is given in Table 8.4. This allows the clinician to monitor the disease during follow-up or to assess the efficacy of an intervention, whether the disease is stable, improving or progressive.

8.12.9 Histopathology

In a majority of OSF specimens, the surface epithelium shows thinning (atrophy) and flattening, while few may show epithelial hyperplasia due to chronic mastication of areca nut. Uniform hyalinization of the juxtaepithelial layer is a pathognomonic feature (Hamner 1974) [103]. Varying degrees of inflammation in the lamina propria with a sprinkling of lymphocytes is found. The characteristic feature is the presence of collagen in the upper part of submucosa [104]. In the early stages, plump fibroblasts may be found. The collagen bundles may extend up to striated muscle and sometimes embed within muscle fibres. The disease can be staged by the state of fibrosis seen in histological sections. However, no correlation has been noted between the clinical stage and the stage of fibrosis, as the biopsy may not be representative [105, 106]. In the early stage, the fibrosis is confined to the upper portion of submucosa. In the intermediate stage, there is subepithelial hyalinization

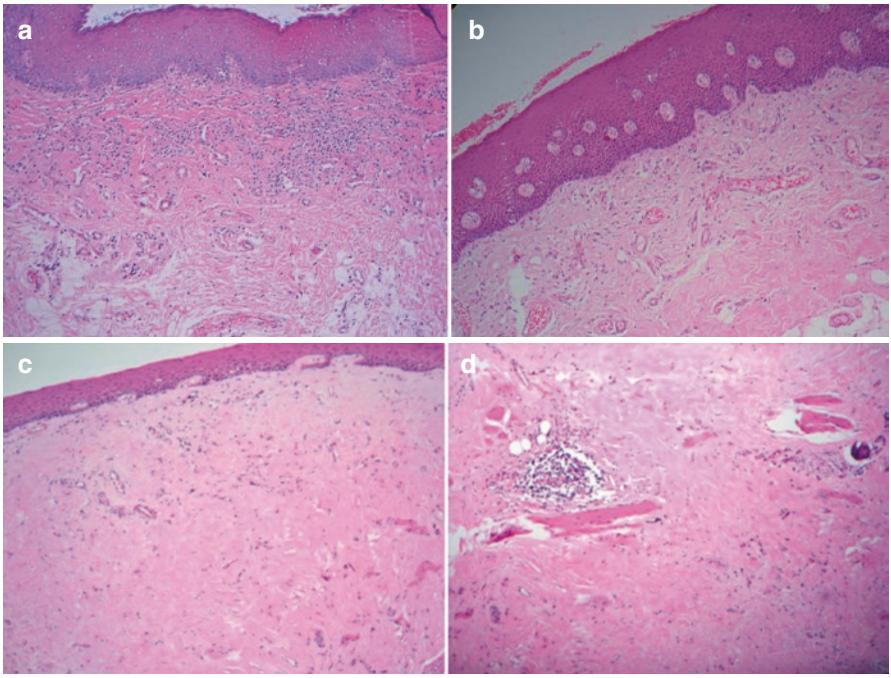

Fig. 8.9 Histopathological features of OSF. (**a**) Early stage: mild atrophy of the epithelium and fibrosis of the upper corium. Light scattered lymphocytic infiltrate within collagen fibres. (**b**) Intermediate stage: fibrosis advances into deeper corium. (**c**) Advanced stage: marked epithelial atrophy and dense fibrosis and hyalinization of the corium and replacement of muscle by fibrous tissue. (**d**) Complete replacement of muscle by fibrous tissue at the advanced stage

with fibrosis extending to deeper tissues. The advanced stage is demarcated with extensive full-thickness fibrosis of the submucosal tissue up to muscle layers together with hyaline changes [86]. Figure. 8.8 illustrates features of fibrosis as noted in the proposed staging by these authors.

In electron microscopic studies, excessive increase of collagen, especially type 1 (van Wyk et al. 1990) [107], and some necrosis of muscle have been reported [108].

Pindborg et al. (1970) [9] first reported the presence of epithelial dysplasia in a quarter of his OSF cases. In a recent study that analysed 42 OSF cases from Sri Lanka, 19 (45.2 %) showed epithelial dysplasia [64]. Figure 8.9 illustrates features of epithelial dysplasia in OSF. No significant association of presence or absence of dysplasia with the stage of fibrosis has been reported, suggesting the two processes are independent of each other. Although it has been proposed that the severity of epithelial dysplasia is proportional to the risk of subsequent cancer development [109], this has yet to be substantiated. Squamous cell carcinoma arsing from

8.12.10 Molecular Studies

Although the exact mechanisms are not clear, various chromosomal, genetic and molecular alterations are associated with the pathogenesis of OSF. An understanding of molecular events in OSF is emerging. One of the earliest attempts to characterise molecular aberrations in OSF related to detailed examination of mutations of P53 gene and its protein expression [110]. Positive p53 immunostaining was observed in 75 % of OSF cases and by PCR-SSCP novel mutations in p53 were reported in exons 2–9. In this study, 16 different mutations in p53 were found in 21 OSF samples from Karachi, Pakistan. Other key molecular features of OSF have recently been described. MMP-1 expression is reportedly attenuated in OSF while TGF-β1 expression is upregulated [64]. MMP-1 is the main human enzyme that degrades fibrillar collagen. As expected, MMP-1 levels in OSF connective tissue were attenuated compared to normal oral mucosa [75]. This shows that collagen degradation caused by MMP-1 is downregulated in the OSF, causing accumulation of ECM in the connective tissue. TGF-β is a known potent mediator which stimulates collagen and other ECM production [86]. Significantly increased TGF-β1 expression has been demonstrated in the lamina propria of OSF compared to NOM. A study using oligonucleotide microarray analysis has shown an upregulation of 716 genes and downregulation of 149 genes in OSF [111]. These genes are involved in the immune response, the inflammatory response and epithelial–mesenchymal transition (EMT) induced by TGF-β signalling pathway, namely, SFRP4, THBS1, MMP2 and ZO-1. In another study, differentially expressed genes in OSF were analysed using bioinformatic tools, and the genes were located on chromosomes 1, 2, 5, 6, 7, 11 and 12. Gene ontology (GO) classification identified these genes to be related to cellular component subgroups associated with extracellular matrix, cytoskeleton and cell membrane and also biological process subgroups associated with protein binding, signal transducer activity and immune and defence responses [112] (Figs. 8.10 and 8.11).

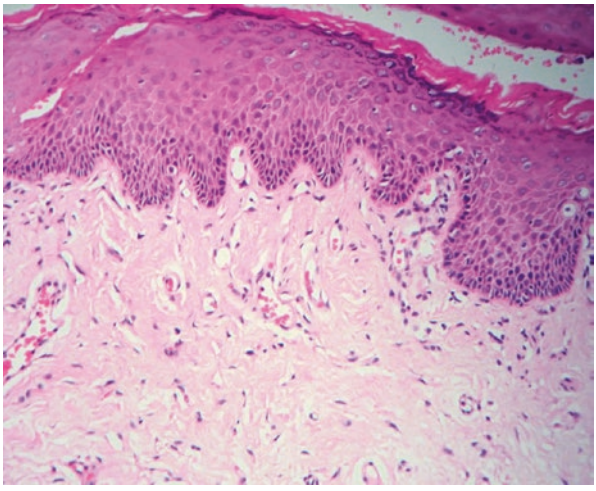

Fig. 8.10 Mild epithelial dysplasia in the background of OSF with new blood vessel formation

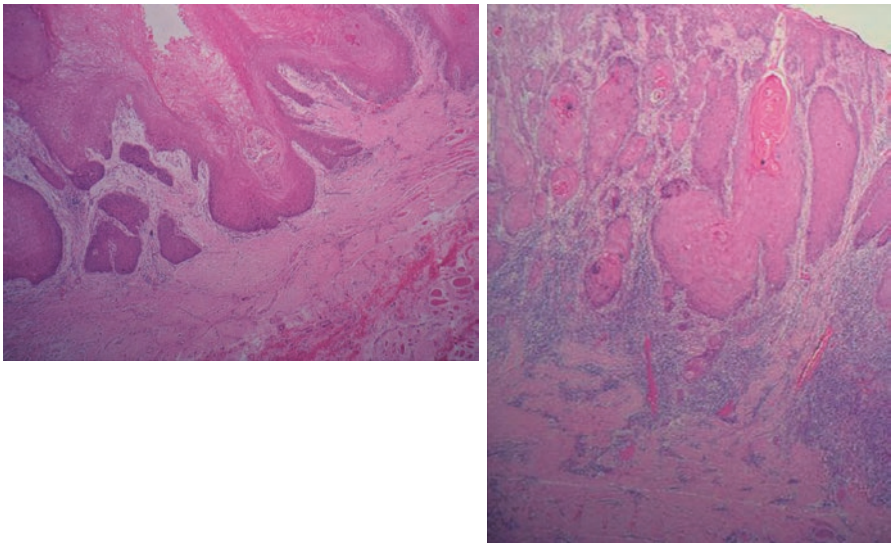

Fig. 8.11 Squamous cell carcinoma in the background of OSF

8.12.11 Malignant Transformation

The observations by Paymaster and Jens Pindborg over 50 years ago claimed the premalignant nature of OSF and provided evidence for its propensity for malignant transformation. Paymaster [6] described the development of slow-growing squamous cell carcinomas in one-third of OSF cases seen at Tata Memorial Hospital in Bombay, India. Pindborg on the other hand was observant of the coexistence of OSF in 40 % of 100 consecutive OSCC cases he reported from south India (Pindborg et al. 1966) [8].

Cancers arising in OSF are noted to be large exophytic lesions which are clinically typical OSCC without showing much histological evidence of invasion. One study reported that most of these patients are younger males showing good prognostic factors: better grades of tumour differentiation, lower rates of nodal metastases and limited extracapsular spread compared to older patients [113]. A retrospective study in China has reported contradictory data in which they state that OSCC originated from OSF is clinically more invasive and also exhibits higher rates of metastasis and recurrence rate than OSCC not originated from OSF [114].

8.12.12 Molecular Events During OSF-Carcinoma Sequence

Epithelial–mesenchymal transition (EMT) is a key mechanism in carcinogenesis. EMT has gained significant attention due to its implication in cancer and fibrosis. TGF-beta may play significant effect on EMT. Cell injury caused by areca nut extract (ANE) produces reactive oxygen species (ROS) which in turn triggers both MAPK and NF-κB pathways involved in EMT of OSF [115]. A study from our

group showed that arecoline upregulates αvβ6 expression in oral keratinocytes which in turn promotes keratinocyte migration and induces invasion [67]. It has been reported that over 80 % of OSCCs arising on a background of OSF had moderate to high αvβ6 expression [67]. We also found a statistically significant correlation with the degree of epithelial dysplasia and expression level of the gene HIF-1α that led us to conclude that hypoxia together with overexpression of HIF-1α play a role in malignant transformation of OSF [116].

Matrix metalloproteinase-2 (MMP-2) can degrade extracellular matrix and basement membrane and play an important role in the development and progression of multiple carcinomas. Subjects carrying CC genotype – a polymorphism in the MMP-2 – had nearly twofold increased risk for developing OSCC when comparing with CT or TT genotype [117].

Genomic instability in the form of LOH has been reported in OSF. This acquisition of LOH may subsequently alter gene function and expression. Several hot spots affecting LOH loci (in 47–53 % of OSF samples) have been identified, and a key finding is LOH in a large region of the chromosome 13-13q14 to 13q33. Considering the well-known fact that chromosome 13q is highly susceptible to genomic instability in HNSCC, we hypothesised that genes within the 13q14–q33 LOH region found in the OSF may play an essential role in the initiation of oral carcinogenesis in these patients. Other LOH loci revealed in this study with previously identified susceptibility regions in HNSCC include 3p24-p22, 6q26-q27, 9q22.3, 12p11.2 and 20p12-11 [118].

8.13 Management

Numerous medical interventions have been tested, but none so far have predictably shown any clinically meaningful benefit in improving mouth opening and other secondary end points. These clinical interventions were discussed at the World Workshop in Oral Medicine V and reported by Kerr et al. [15]. A wide range of medical interventions have been studied and include nutritional supplements, antioxidants, anti-inflammatory and immunomodulatory agents, biogenic stimulators, cytokines, enzymes and fibrinolytic agents and vasodilators.

Nutritional supplements (i.e. vitamin A, vitamin B, multivitamins, iron, zinc) and antioxidants (i.e. lycopene, beta-carotene, tea pigments, aloe vera and curcumin) are thought to correct deficiency states, promote tissue health and reduce the propensity for adverse effects secondary to chronic areca nut use. Anti-inflammatory and immunomodulatory agents (i.e. topical and intralesional corticosteroids, interferon gamma (IFNγ), levamisole) are thought to reduce the pro-fibrotic inflammatory pathways. Intralesional placental extracts have been hypothesised to act as biogenic stimulators, promoting regeneration of healthy tissues. Enzymes and fibrinolytic agents (i.e. hyaluronidase, collagenase and chymotrypsin) have been tested to degrade fibrotic tissues. Finally, vasodilators (i.e. pentoxifylline, nylidrin hydrochloride and buflomedil hydrochloride) have been tested to boost blood flow to the tissues. Many of these interventions have been tested in uncontrolled open label studies, and the randomised controlled trials on OSF that have been conducted so

far, have significant limitations. Most non-specific antifibrotic agents used as therapeutic regimes have been ineffective in halting or reversing fibrosis.

A few studies, with variable results, have explored the use of physiotherapy, either alone or as an adjunct to medical and surgical therapies to increase opening through tissue remodelling.

For advanced cases various surgical treatments with numerous different types of flap reconstruction have been reported. Immediate outcome are generally excellent, although there is often relapse in mouth opening. Physiotherapy undertaken immediate post-surgery could sustain the noted improvement. Finally, there is very little research exploring the impact of habit cessation on these interventions.

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