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THE MULTISTEP CARCINOGENIC JOURNEY FROM ORAL MUCOSA TO FIBROSIS TO CARCINOMA: INTEGRATING GENOMIC EVOLUTION, MICROENVIRONMENT REMODELING, AND STEM CELL DYNAMICS

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ABSTRACT

Oral submucous fibrosis (OSF) is a chronic, progressive, and potentially malignant disorder of the oral mucosa strongly associated with areca nut chewing. Despite decades of research, OSF remains a major public health challenge in South and Southeast Asia, where its prevalence continues to rise. Malignant transformation to oral squamous cell carcinoma (OSCC) is the most serious consequence, with reported rates ranging from 3% to 19%. This review synthesizes current evidence on the cellular, molecular, and epidemiological factors that drive this transition. Chronic exposure to arecoline and other alkaloids induces oxidative stress, activates the TGF-β/SMAD signaling pathway, and promotes excessive collagen deposition, creating a hypoxic and fibrotic microenvironment. Over time, persistent epithelial atrophy and inflammation foster genetic and epigenetic alterations including TP53 mutation, loss of p16, and dysregulation of microRNAs that destabilize cell-cycle control and DNA repair. Histopathologically, this is reflected by progressive epithelial dysplasia and eventual invasion of malignant keratinocytes. Epidemiological studies consistently highlight duration and frequency of areca nut use, concurrent tobacco exposure, and high-grade fibrosis as key predictors of transformation. Understanding these pathways is critical for developing reliable biomarkers, refining risk stratification, and guiding early detection strategies. A clearer grasp of the molecular crosstalk between fibrogenesis and carcinogenesis will inform targeted prevention and therapeutic interventions aimed at interrupting the OSF–OSCC continuum.

Keywords: *Oral submucous fibrosis, Oral squamous cell carcinoma, Malignant transformation, Areca nut, Arecoline, TGF-β signaling, Hypoxia, Fibrosis, Epithelial dysplasia, Molecular pathogenesis, Biomarkers, Carcinogenesis*

INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents one of the most common malignancies worldwide, accounting for approximately 90% of all oral cancers and posing a significant global health burden with high morbidity and mortality rates. Despite advances in diagnostic techniques and therapeutic modalities, the five-year survival rate for OSCC remains disappointingly low, primarily due to late-stage diagnosis, frequent recurrence, and development of treatment resistance. (1) The stepwise progression of OSCC offers a unique window to study carcinogenesis: from normal oral mucosa → potentially malignant disorders → frank carcinoma.

This progression frequently originates from oral potentially malignant disorders (OPMDs), which undergo malignant transformation through the accumulation of genetic and epigenetic alterations. Among these OPMDs, oral submucous fibrosis (OSF) occupies a central role due to its high malignant transformation rates, ranging from 3–10%, with variability across populations, particularly in South and Southeast Asia where betel quid chewing is endemic (2-4) OSF is a chronic, insidious, and progressive condition characterized by epithelial atrophy, excessive collagen deposition, progressive fibrosis of the lamina propria and submucosa, and eventual epithelial dysplasia, setting the stage for OSCC development.

The pathogenesis of OSCC represents a complex multistep process involving the interplay of genomic instability, microenvironmental remodeling, and cellular plasticity (5). This review aims to synthesize current understanding of the pathological continuum from normal oral mucosa through fibrotic transformation to invasive carcinoma, with particular emphasis on OSF as a critical precursor lesion. We will explore how chronic exposure to risk factors initiates a cascade of molecular events that disrupt epithelial homeostasis, promote stromal fibrosis, and ultimately create a permissive environment for carcinogenesis. The cancer stem cell (CSC) paradigm will be examined as a critical element in this process, providing insights into the mechanisms underlying therapy resistance and tumor recurrence .

Recent advances in genomic technologies have revolutionized our understanding of OSCC evolution, revealing remarkable intratumor heterogeneity and clonal dynamics that shape disease progression and treatment response. Simultaneously, growing appreciation of the tumor microenvironment (TME) has highlighted the importance of bidirectional crosstalk between neoplastic cells and various stromal components, including immune cells, fibroblasts, and vascular elements, in driving OSCC pathogenesis. This review will integrate these multifaceted perspectives to provide a comprehensive overview of the molecular and cellular events that characterize the journey from normal mucosa to OSF to OSCC, while discussing implications for early detection, risk stratification, and targeted therapeutic intervention .

NORMAL ORAL MUCOSA:

The oral mucosa, the specialized mucous membrane lining the oral cavity, represents a critical interface between the external environment and the internal body systems, serving as a dynamic and multifunctional tissue essential for overall health. (6, 7) Embryologically, it is derived primarily from ectoderm, with contributions from endoderm in the pharyngeal region, and its development is governed by complex epithelial-mesenchymal interactions that dictate its regional specialization. (8) This tissue is not a homogeneous structure but is anatomically and histologically differentiated into three main types based on functional demands: the masticatory mucosa (gingiva and hard palate), which is designed to withstand shear force and abrasion; the lining mucosa (lips, cheeks, floor of the mouth, soft palate), which provides elasticity and pliability for speech and mastication; and the specialized mucosa (dorsal tongue), which is uniquely adapted for taste sensation. (9) The integrity of this organ is paramount, as it performs a vast array of functions, including mastication, speech, sensation, and, most critically, serving as the body's first line of immunological defense, all while

maintaining a symbiotic relationship with a diverse community of commensal microorganisms known as the oral microbiome. (7, 10)

Histological Structure: A Layered Defense System

The architecture of the healthy oral mucosa is a masterclass in functional design, consisting of two primary layers: a stratified squamous epithelium and an underlying supportive connective tissue layer known as the lamina propria. The oral epithelium is a highly dynamic tissue with a rapid turnover rate of approximately 14-21 days, facilitated by progenitor stem cells residing in the stratum basale, the deepest layer. (11) The nature of this epithelium varies regionally to meet functional needs. In masticatory areas, it is keratinized, comprising a stratum basale, stratum spinosum (where cells are densely connected by desmosomes), stratum granulosum (containing keratohyalin granules), and a superficial stratum corneum of anucleate, keratin-filled cells that provide a tough, protective barrier. In contrast, the lining mucosa is non-keratinized, featuring a stratum basale, stratum spinosum, and a superficial stratum superficiale of nucleated cells with dispersed keratin bundles, allowing for the necessary flexibility and comfort. (12) Beneath this epithelium lies the lamina propria, a dense fibrous connective tissue layer divided into a superficial, loose papillary layer that projects into the epithelium as connective tissue papillae, increasing the surface area for adhesion and nutrient transfer and a deeper reticular layer with thicker collagen bundles. (11, 13) This entire structure is anchored to a sophisticated basement membrane, an ultra-structural complex of proteins like laminin and type IV collagen that provides not only physical attachment but also a semi-permeable filter and a crucial signaling interface between the epithelial and connective tissue compartments. In some regions, a deeper submucosa containing larger blood vessels, nerves, and minor salivary glands provides additional cushioning and secures the mucosa to underlying bone or muscle. (12, 14)

Functions of the Oral Mucosa

The protective function of the healthy oral mucosa is a multifaceted endeavor achieved through a combination of physical, chemical, and sophisticated immunological mechanisms. (14) The physical barrier is formed by the tightly interconnected epithelial cells and, where present, the keratinized surface, which prevents the penetration of microbes and toxins. This is powerfully complemented by the chemical environment; the oral mucosa is constantly bathed in saliva, which provides a washing action and contains a potent arsenal of antimicrobial agents, including lysozyme, lactoferrin, peroxidase, and antimicrobial peptides like defensins and histatins. (7, 15) Furthermore, saliva secretes mucins, high-molecular-weight glycoproteins that form a protective, lubricating layer over the mucosal surface, trapping pathogens and facilitating their clearance. The most advanced level of protection is offered by the mucosa's highly developed immune surveillance system. This system features resident antigen-presenting cells, most important Langerhans cells in the epithelium, which capture foreign antigens and migrate to initiate adaptive immune responses. (11) It also includes intraepithelial lymphocytes (IELs) that provide rapid, localized defense and a population of plasma cells in the lamina propria that secrete immunoglobulin A (IgA). Secretory IgA (sIgA) is the predominant antibody in mucosal defense, capable of neutralizing pathogens and preventing their adhesion to the epithelial surface without provoking a damaging inflammatory response. (6, 7) This entire defense apparatus operates in a state of careful balance, maintaining tolerance to the trillions of commensal bacteria that constitute the oral microbiome while remaining poised to mount a robust defense against true pathogens, a relationship known as homeostasis (6).

ORAL SUBMUCOUS FIBROSIS (OSF):

Oral submucous fibrosis (OSF) is a chronic, progressive disorder characterized by the abnormal deposition of collagen in the submucosal tissues of the oral cavity, leading to progressive stiffness and reduced mobility

of the affected structures. (16) First described in the early 1950s, OSF has since been recognized as a potentially malignant disorder with transformation rates ranging from 2.6% to 13% depending on geographical regions and population studies (3, 17-21). The condition predominantly affects individuals of Asian descent, particularly those from the Indian subcontinent, Southeast Asia, and the Pacific Islands, though global migration patterns have spread its prevalence to other parts of the world. (22, 23)

The clinical presentation of OSF evolves through various stages, beginning with symptoms such as burning sensation when consuming spicy foods, blanching of the oral mucosa, and gradual development of fibrous bands that ultimately restrict mouth opening (trismus). As the disease progresses, patients may experience xerostomia (dry mouth), dysphagia (difficulty swallowing), and reduced tongue mobility, significantly impairing speech, nutrition, and overall quality of life. (24, 25) The condition typically affects multiple oral sites, with the buccal mucosa, retromolar area, soft palate, and faucial pillars being most commonly involved. In advanced stages, the fibrosis may extend to the pharynx and upper third of the esophagus, creating profound functional limitations. (24)

Epidemiology

The global prevalence of OSF demonstrates striking geographical variations closely tied to cultural practices and substance consumption patterns. According to recent epidemiological data, India has witnessed a dramatic increase in OSF cases, with prevalence rates rising from approximately 0.03% to 6.4% over the past four decades. (26, 27) Certain regions within India report exceptionally high rates, reaching 30-42% in northern states, while western areas show lower prevalence (0.03-0.2%). (28) Other Asian countries significantly affected include Taiwan (0.086-17.6%), Vietnam (0.15-14.6%), China (0.9-4.7%), and Sri Lanka. (29) The condition demonstrates a slight male predilection in most regions (0.2-2.3% in males versus 1.2-4.57% in females), though this distribution varies geographically. (30) Of particular concern is the increasing incidence among younger populations, including adolescents and young adults, attributed to the widespread availability of commercially prepared areca nut products .

Etiology:

The etiology of OSF is multifactorial, involving a complex interplay between primary environmental triggers, genetic predisposition, and secondary contributing factors. The transformation from healthy oral mucosa to a fibrotic state is not initiated by a single agent but rather by a confluence of these elements that disrupt tissue homeostasis and activate a pathological fibroproliferative cascade (fig1) (3, 16).

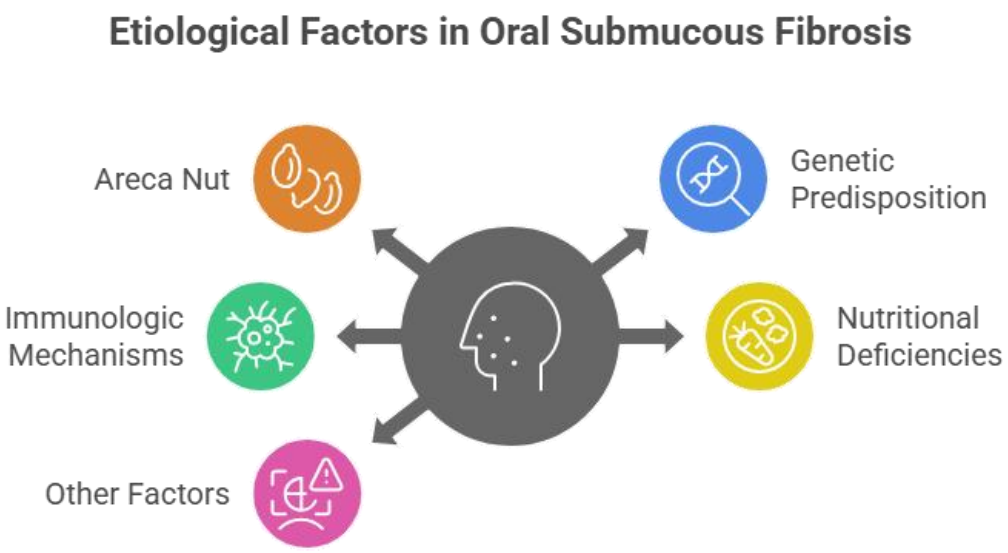


FIGURE 1: ETIOLOGICAL FACTORS IN OSF

1. **Areca Nut: The Primary Etiological Agent**

The consumption of areca nut (from the Areca catechu palm) is unequivocally established as the primary and most significant causative factor for OSF. Its causal role is supported by extensive epidemiological evidence, clinical studies, and in vitro experiments. (26) The International Agency for Research on Cancer (IARC) has classified the areca nut as a Group 1 carcinogen (carcinogenic to humans). The pathogenicity of the nut is not due to a single component but rather a combination of its alkaloids, tannins, and physical properties.The four main alkaloids (arecoline, arecaidine, guvacine, and guvacoline) are central to the fibrotic process.

- **Arecoline:** It is the most abundant and studied alkaloid. It is hydrolyzed in saliva to arecaidine. Both compounds directly stimulate fibroblasts to proliferate and synthesize abnormally high amounts of collagen (types I and III) and extracellular matrix (ECM) proteins. They also inhibit enzymes like collagenase and other matrix metalloproteinases (MMPs) that normally break down collagen, creating an imbalance that favors accumulation. (31, 32) Arecoline induces the generation of Reactive Oxygen Species (ROS), leading to oxidative stress and DNA damage. It also upregulates key pro-fibrotic cytokines, most notably Transforming Growth Factor-beta (TGF-β), which is the master switch for fibrosis. TGF-β signaling promotes the differentiation of fibroblasts into highly active myofibroblasts that are resistant to apoptosis. (31)
- **Tannins (Polyphenols):** Areca nut is rich in condensed tannins (15-20%). Tannins act as potent protein binders. They increase the cross-linking of collagen fibers, making them more stable and resistant to enzymatic degradation. This directly contributes to the irreversible rigidity of the tissues. (33)
- **Copper:** Commercial processing and storage of areca nuts in copper vessels or the inherent high copper content in the nut itself lead to significantly elevated copper levels in the tissue. Copper is a crucial co-factor for the enzyme lysyl oxidase (LOX). LOX catalyzes the cross-linking of collagen and elastin fibers, further stabilizing the collagen matrix and making it insoluble. Studies consistently show elevated serum and salivary copper levels in OSF patients compared to healthy controls. (34)
- **Physical Trauma:** The coarse, fibrous nature of the areca nut causes constant

microtrauma and mechanical irritation to the oral mucosa, particularly in the buccal mucosa and retro-molar areas where the quid is typically placed. This repeated injury disrupts the epithelial barrier, facilitating the deeper penetration of alkaloids and initiating a chronic cycle of inflammation and repair that progresses to fibrosis. (9, 32)

2. Genetic Predisposition

The fact that only a subset of areca nut users develop OSF suggests a strong role for genetic susceptibility. Certain individuals possess genetic polymorphisms that make them more vulnerable to the toxic effects of areca nut constituents.

- **Cytokine Gene Polymorphisms:** Variations in the genes encoding pro-fibrotic (TGF-β1, TNF-α) and anti-fibrotic (IFN-γ) cytokines can alter an individual's immune response, predisposing them to a heightened fibrotic reaction. (35)
- **Matrix Remodeling Gene Polymorphisms:** Genes responsible for collagen degradation (e.g., MMP-1, MMP-3, MMP-9) and their inhibitors (TIMP-1, TIMP-2) can be polymorphic. An imbalance favoring TIMPs over MMPs creates an environment where collagen synthesis outstrips its breakdown.
- **HLA Association:** Certain Human Leukocyte Antigen (HLA) haplotypes have been found to be more prevalent in OSF patients, indicating a possible immune-mediated genetic susceptibility.

3. Immunologic and Inflammatory Mechanisms

Chronic irritation from quid chewing creates a persistent inflammatory milieu:

- Infiltration by T-lymphocytes and increased Th1 cytokines (IFN-γ, TNF-α, IL-1β) stimulate fibroblast proliferation and collagen synthesis.
- Up-regulation of TGF-β, a key fibrogenic cytokine, drives fibroblast-to-myofibroblast differentiation and excessive extracellular matrix deposition. This cytokine imbalance explains why fibrosis continues even after quitting the habit in some patients. (36)

4. Nutritional Deficiencies

Nutritional deficiencies are considered important secondary or aggravating factors rather than a primary cause. They likely reduce the mucosal resistance to local irritants and impair the ability to repair sub-clinical damage.(37)

- **Iron Deficiency (Anaemia):** Iron is a co-factor for enzymes involved in epithelial maturation and maintenance. Its deficiency can lead to epithelial atrophy, making the mucosa more susceptible to the penetration of areca nut alkaloids. (37)
- **Vitamin B Complex Deficiency:** Vitamins B, particularly B12, B6, and folate, are crucial for cell division, DNA synthesis, and overall mucosal health. Their deficiency can exacerbate epithelial damage and impair healing. (37)
- **Antioxidant Deficiency:** A diet deficient in antioxidants (Vitamins A, C, E) compromises the body's defense against the oxidative stress generated by areca nut chewing, allowing for greater cellular damage. (37)

4. Other Proposed Factors

- **Autoimmunity:** Some theories propose that certain components of the areca nut may trigger an autoimmune response against connective tissue proteins (e.g., collagen), leading to a chronic inflammatory and fibrotic process. The presence of autoantibodies in some OSF patients supports this hypothesis. (6, 7)
- **Chillies and Spicy Food:** The capsaicin in chillies can cause irritation, inflammation, and epithelial damage. While not a primary cause, it may act as a synergistic irritant that exacerbates the effects of areca nut. (37)
- **Microbial Agents:** Chronic candidal infections are frequently observed in OSF, though they are likely a consequence of the stagnant mucosal folds and epithelial atrophy rather than a cause. However, the chronic

inflammation from such infections may contribute to the progression of fibrosis. (15)

TABLE 1: ETIOLOGICAL FACTORS AND THEIR KEY MOLECULAR MECHANISMS IN THE PATHOGENESIS OF OSF

Etiological Factors	Key Components / Mechanisms	Pathogenic Role in OSF
Areca Nut (Primary Agent)	**Alkaloids** (arecoline, arecaidine, guvacine, guvacoline)	Stimulate fibroblast proliferation and excess collagen (types I & III) synthesis; inhibit collagenase/MMPs; generate ROS → oxidative stress and DNA damage; up-regulate TGF-β, driving fibroblast-to-myofibroblasts differentiation
	Tannins (Polyphenols)	Increase collagen cross-linking and resistance to enzymatic degradation, causing irreversible fibrosis
	Copper	Elevated copper enhances lysyl oxidase (LOX) activity → cross-linking of collagen and elastin → stabilized, insoluble collagen
	Physical Trauma	Coarse nut fibers cause chronic mucosal microtrauma, facilitating deeper penetration of alkaloids and perpetuating inflammation
Genetic Predisposition	Cytokine polymorphisms (TGF-β1, TNF-α, IFN-γ)	Alter immune/fibrotic response, predisposing to excessive fibrosis
	Metabolizing enzyme polymorphisms (GST M1/T1, CYP450)	Reduce detoxification of alkaloids and ROS, enhancing tissue damage
	Matrix remodeling genes (MMP-1, MMP-3, MMP-9, TIMPs)	TIMP dominance limits collagen degradation, favoring accumulation
	HLA haplotypes	Certain HLA types linked to increased susceptibility via immune dysregulation
Immunologic & Inflammatory Mechanisms	Chronic T-cell infiltration, elevated IFN-γ, TNF-α, IL-1β	Persistent inflammation stimulates fibroblast proliferation and collagen deposition
	Upregulation of TGF-β	Central driver of fibroblast activation and extracellular matrix accumulation, sustaining fibrosis even after habit cessation
Nutritional Deficiencies	Iron deficiency	Epithelial atrophy increases mucosal vulnerability to areca alkaloids
	Vitamin B complex deficiency (B12, B6, folate)	Impaired DNA synthesis and epithelial repair
	Antioxidant deficiency (vitamins	Reduced defense against ROS, amplifying oxidative damage

	A, C, E)	
	Autoimmunity	Possible autoantibodies against collagen perpetuate fibrosis
Other Factors	Chillies/spicy foods	Capsaicin induces mucosal irritation and synergizes with areca nut effects
	Microbial agents (e.g., Candida)	Secondary infections maintain chronic inflammation and may promote fibrosis

Pathogenesis:

The transformation from normal mucosa to fibrotic tissue involves complex cellular interactions between epithelial cells, fibroblasts, immune cells, and endothelial cells. This pathogenesis can be conceptualized in several key stages: (5, 27)

1. **Initial Contact: Chemical and Mechanical Irritation**

The transformation begins when the oral mucosa is repeatedly exposed to areca nut alkaloids (arecoline, arecaidine), polyphenols, slaked lime, and often tobacco. (24, 32, 34) Chewing releases these agents in an alkaline environment that enhances absorption and generates reactive oxygen species (ROS). At the same time, the coarse, fibrous texture of the nut causes microtrauma to the mucosa. This combination of chemical irritation and physical injury disrupts epithelial cell membranes, induces oxidative stress, and activates basal keratinocytes to release pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). (38)

2. **Early Epithelial and Sub-mucosal Changes**

In response to repeated insult, the oral epithelium shows basal cell hyperplasia and occasional spongiosis, while the underlying lamina propria develops vascular dilatation and edema. (21, 26) Inflammatory cells, mainly lymphocytes and macrophages accumulate in the submucosa and begin secreting transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF), and other mediators that prime resident fibroblasts for activation. (35)

3. **Inflammation and Cytokine Release**

The tissue damage and cellular stress act as a potent trigger for a chronic inflammatory response. This is characterized by the infiltration of lymphocytes, macrophages, and other immune cells into the submucosa. (2, 35, 38) These activated inflammatory cells release a flood of pro-inflammatory and pro-fibrotic cytokines:

- **Transforming Growth Factor-Beta (TGF-β):** The master regulator of fibrosis. TGF-β is massively upregulated in OSF and is the key driver of the entire process. (31, 35)
- **Tumor Necrosis Factor-Alpha (TNF-α), Interleukin-1 (IL-1), Interleukin-6 (IL-6):** Promote inflammation and stimulate fibroblast proliferation. (38)
- **Platelet-Derived Growth Factor (PDGF), Connective Tissue Growth Factor (CTGF):** Potent mitogens and activators of fibroblasts. (35)

This cytokine storm creates a pro-fibrotic microenvironment, signaling the resident cells to initiate a repair process that goes awry.

4. **Fibroblast Activation and Myofibroblast Differentiation**

Under the influence of the sustained cytokine signal, particularly TGF-β, the resident fibroblasts in the lamina propria undergo a dramatic transformation. (35, 38)

- **Proliferation:** Fibroblasts proliferate rapidly, increasing their population. (38)
- **Activation and Differentiation:** They differentiate into myofibroblasts, the "effector cells" of fibrosis. These cells express α-smooth muscle actin (α-SMA), which gives them contractile properties, and they are highly synthetic. (4, 35)
- **Excessive ECM Production:** Activated myofibroblasts embark on a pathological production of ECM components, chiefly collagen types I and III, fibronectin, and elastin. The rate of collagen synthesis far exceeds that in normal mucosa. (35, 38)

5. **Disruption of Collagen Degradation**

Normally, collagen turnover is regulated by a balance between matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). In

OSF, arecoline and TGF-β suppress MMP expression while elevating TIMPs, creating an environment where collagen degradation is sharply reduced. (32, 38) Polyphenols and tannins from areca nut add another layer of stability by directly cross-linking collagen fibers. The result is progressive extracellular matrix accumulation within the lamina propria and submucosa. (31, 35)

6. **Collagen Stabilization and Epithelial Atrophy**

As collagen bundles thicken and contract, they compress local blood vessels, leading to ischemia and chronic hypoxia. (38) Reduced perfusion limits nutrient delivery to the overlying epithelium, causing epithelial atrophy and loss of elasticity. Hypoxia stabilizes hypoxia-inducible factor-1α (HIF-1α), which paradoxically promotes angiogenic signals but cannot overcome the mechanical barrier of dense collagen. Clinically, this corresponds to a pale, stiff mucosa with loss of pliability. (39)

7. **Formation of Fibrous Bands and Trismus**

With ongoing collagen deposition and cross-linking, the submucosa becomes fibrotic and inelastic. Palpable fibrous bands develop in the buccal mucosa, soft palate, and retromolar region. (24) Contraction of these collagen bundles, along with involvement of the pterygomandibular raphe and masticatory muscles, produces restricted mouth opening (trismus) and a characteristic "wooden" texture of the oral mucosa. (24, 26)

8. **Establishment of a Premalignant Microenvironment**

The chronic inflammatory milieu and persistent oxidative stress create fertile ground for genetic and epigenetic alterations. ROS and nitrosamines from areca nut and tobacco cause DNA damage, while chronic TGF-β signaling can shift from growth-inhibitory to pro-oncogenic, encouraging epithelial–mesenchymal transition (EMT). (31, 32, 35) Mutations in p53, promoter hypermethylation of tumor suppressor genes (e.g., p16), and altered microRNA profiles have all been documented. These molecular changes explain the markedly increased risk of OSCC in longstanding OSF. (3, 38)

TABLE 2: SEQUENTIAL CELLULAR AND MOLECULAR MECHANISMS DRIVING THE PROGRESSION OF OSF

Stage	Cellular Players	Molecular/Pathological Changes	Clinical Correlate
Chemical & Mechanical Irritation	Areca nut alkaloids (arecoline, arecaidine), polyphenols, slaked lime, tobacco	ROS generation, oxidative stress, epithelial membrane disruption, release of IL-1β, TNF-α	Burning sensation, mild mucosal erythema or roughness
Epithelial & Submucosal Changes	Basal keratinocytes, endothelial cells, resident fibroblasts	Basal cell hyperplasia, vascular dilatation, edema, early infiltration of lymphocytes/macrophages	Mucosal thickening, early blanching
Inflammation & Cytokine	Lymphocytes, macrophages, epithelial cells	Surge of TGF-β (master pro-fibrotic), TNF-α, IL-1, IL-6, PDGF, CTGF → strong fibroblast activation signals	Persistent burning, early stiffness
Fibroblast Activation & Myofibroblast Differentiation	Fibroblasts → myofibroblasts (α-SMA positive)	Rapid fibroblast proliferation; differentiation to contractile, ECM-producing myofibroblasts; excessive collagen I/III,	Palpable thickening of mucosa

		fibronectin production	
Disruption of Collagen Degradation	Fibroblasts, ECM enzymes	Suppression of MMPs, up-regulation of TIMPs; polyphenol-induced collagen cross-linking → reduced degradation	Progressive induration of buccal mucosa
Collagen Stabilization & Epithelial Atrophy	Collagen bundles, endothelial cells	Dense collagen compresses blood vessels → ischemia, hypoxia; stabilization of HIF-1α; epithelial thinning and atrophy	Pale, leathery mucosa with reduced elasticity
Formation of Fibrous Bands & Trismus	Myofibroblasts, ECM	Further cross-linking and contraction of collagen → formation of fibrous bands involving buccal mucosa, soft palate, pterygomandibular raphe	Progressive mouth opening restriction (trismus), "wooden" texture
Premalignant Microenvironment	Epithelial cells, fibroblasts, immune cells	ROS-induced DNA damage, p53 mutations, p16 promoter hypermethylation, chronic TGF-β signaling promoting EMT, altered microRNA profiles	High risk of OSCC

Histopathological Progression: Stages of Transformation

The transformation from normal oral mucosa to advanced OSF follows a characteristic sequence of histopathological changes that correlate with clinical progression. (27) In the earliest stages, the mucosa appears relatively normal or may show mild erythema and edema due to vascular dilation and increased permeability. The initial histological changes include epithelial atrophy with loss of rete ridges, basal cell hyperplasia with hyperchromatism, and the presence of a diffuse inflammatory infiltrate predominantly composed of lymphocytes, plasma cells, and eosinophils in the submucosa. (40) At this stage, collagen fibers appear edematous and dispersed, with beginning signs of hyalinization. (24)

As the disease progresses to the intermediate stage, the inflammatory infiltrate becomes more pronounced and concentrated in the subepithelial region. The epithelium shows further atrophy with reduced vascularity, and the collagen fibers undergo progressive hyalinization, losing their distinct fibrillar structure and forming homogeneous, eosinophilic masses. (2, 26) Fibroblasts demonstrate increased size and metabolic activity, with some transforming into myofibroblasts characterized by expression of α-SMA. The elastic fibers become fragmented and diminished, contributing to the loss of tissue elasticity characteristic of OSF. (35)

In advanced OSF, the histopathological picture is dominated by extensive hyalinization of the submucosal connective tissue, with thick, dense collagen bundles arranged in various orientations. The epithelium becomes markedly atrophic, often reduced to only a few cell layers thick, with loss of normal maturation patterns and increased risk of malignant transformation. (24, 40) Vascularity is significantly reduced, with obliteration of small blood vessels and capillaries, contributing to tissue ischemia. The inflammatory infiltrate may become less prominent in this stage, though scattered chronic inflammatory cells persist around the hyalinized areas. The muscle fibers in affected areas show degenerative changes

with fragmentation and loss of striations, further contributing to trismus and functional impairment. (16, 26)

The histopathological progression of OSF is accompanied by characteristic molecular changes that reflect the underlying disease processes. There is progressive upregulation of profibrotic genes (TGF-β, CTGF, PDGF) (31, 35) and increased expression of collagen genes (COL1A1, COL3A1). (2) Simultaneously, genes encoding matrix-degrading enzymes (MMP-1, MMP-2, MMP-9) are downregulated, (38, 40) while their inhibitors (TIMP-1, TIMP-2) are upregulated, creating an environment favorable to matrix accumulation. (35, 38) Oxidative stress markers (8-OHdG, malondialdehyde) show progressive increase, while antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) are depleted, reflecting the escalating oxidative damage. (41) These molecular changes not only drive fibrosis progression but also create a microenvironment conducive to malignant transformation through accumulation of genetic damage and impairment of DNA repair mechanisms. (16, 27)

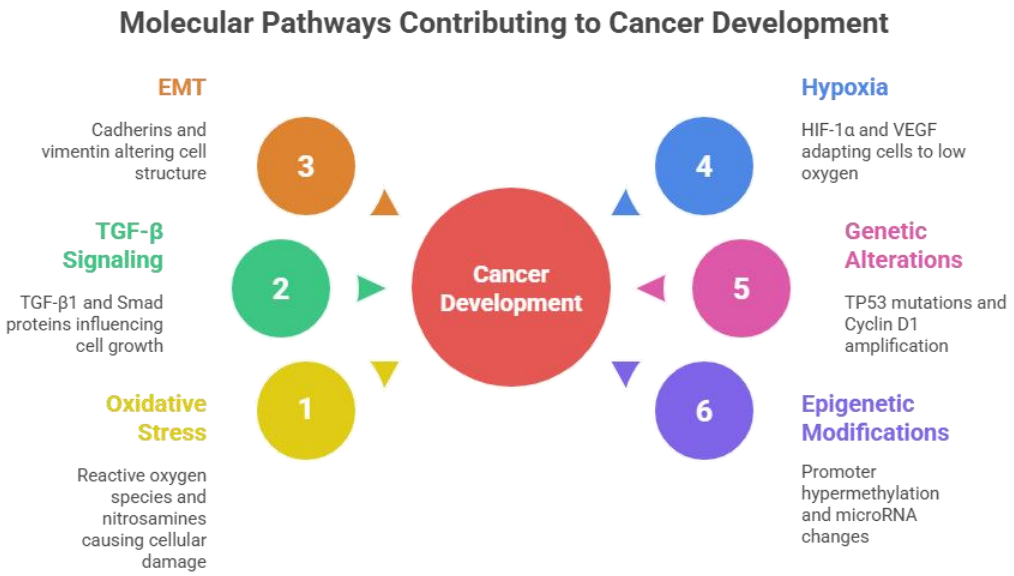
MALIGNANT TRANSFORMATION: FROM FIBROSIS TO CANCER

The progression from OSF to OSCC represents a multistep process involving accumulating genetic and epigenetic alterations that transform fibroblasts and epithelial cells into malignant phenotypes. The overall risk of malignant transformation in OSF ranges from 1.9% to 12% across different populations, (3, 20, 42) though certain high-risk groups demonstrate transformation rates as high as 23%. (43) The risk is significantly elevated when OSF coexists with other potentially malignant disorders, particularly oral leukoplakia (OLK). Studies from Taiwan report malignant transformation rates of 11.1-18.5% for OSF with OLK, compared to 4.6-7.2% for OSF alone. (19) This transformation represents a significant clinical challenge, as OSCC is one of the most common malignancies of the head and neck region, with 377,713 new cases and 177,757 deaths reported globally in 2020 alone. (44)

Transformation is not a single mutation event. Instead, carcinogenesis in OSF follows a multi-hit model in which repeated chemical and mechanical injury (arecoline, polyphenols, slaked lime, tobacco nitrosamines) produces chronic inflammation, oxidative DNA damage, epigenetic reprogramming, and stromal remodeling. (16, 33) The fibrotic stroma itself shapes selection pressures hypoxia, altered matrix stiffness, and paracrine signaling that favor malignant clones. (5, 19, 35) Reviews and mechanistic studies frame TGF-β signaling, ROS generation, EMT induction, and stromal cell reprogramming as central, interacting pathways. (2, 3, 21, 24, 32)

Molecular Pathogenesis of Malignant Transformation

The malignant transformation of OSF into OSCC is a gradual, multistep process characterized by progressive genetic and epigenetic alterations. Chronic exposure to areca nut components, particularly arecoline, sustains a state of persistent inflammation and oxidative stress. (5, 16, 32, 40) This hostile microenvironment generates reactive oxygen species and pro-inflammatory cytokines that induce DNA damage, impair repair mechanisms, and promote chromosomal instability, ultimately leading to aneuploidy. (5, 35, 40) Over time, these cumulative molecular changes disrupt normal regulatory pathways, allowing epithelial cells to escape apoptosis, acquire uncontrolled proliferative capacity, and develop the invasive properties that define malignant transformation (33, 35, 41) (Fig 2).



1. **Oxidative Stress and Genotoxic Damage**

Areca-nut components and tobacco produce reactive oxygen species (ROS) and nitrosamines that damage DNA (base modifications, strand breaks) and lipids, overwhelming repair mechanisms over time. Persistent oxidative injury is repeatedly identified as an early driver of mutagenesis in OSF lesions and a contributor to genomic instability in progressing epithelia. (31, 32, 41, 45)

2. **TGF-β Signaling: Fibrosis and a Switch to Pro-Oncogenic Behavior**

TGF-β occupies a central role in the progression from fibrosis to carcinogenesis. During the early stages of disease, TGF-β acts as a tumor suppressor by inhibiting epithelial cell proliferation and maintaining tissue homeostasis. With chronic stimulation, however, particularly under the influence of arecoline and pro-inflammatory cytokines, TGF-β signaling becomes persistently activated. (45) This sustained activation drives fibroblast proliferation, myofibroblast differentiation, and excessive extracellular matrix deposition and cross-linking through lysyl oxidase in a copper-dependent manner. Over time, prolonged TGF-β activity also induces epithelial–mesenchymal transition (EMT), enhances cellular invasiveness, and reshapes the immune microenvironment, ultimately shifting from a tumor-suppressive to a tumor-promoting function. (5, 35, 38, 45, 46)

3. **EMT, Cellular Plasticity, and Invasion**

EMT transcription factors such as Snail, Slug, and Twist are consistently upregulated in chronic OSF-related lesions and in OSCC that develops within this fibrotic background. (46) EMT is characterized by reduced epithelial adhesion through the loss of E-cadherin, increased expression of mesenchymal markers including N-cadherin and vimentin, and the acquisition of cellular motility and invasive potential. (47) This process is activated by a convergence of signaling pathways involving TGF-β, hypoxia with stabilization of HIF-1α, pro-inflammatory cytokines, and dysregulated microRNA expression, collectively fostering a cellular phenotype that is highly susceptible to invasion and metastatic dissemination. (5, 39, 47)

4. **Hypoxia and Metabolic Adaptation**

Dense, extensively cross-linked collagen in the submucosa compresses local blood vessels and creates a state of chronic hypoxia. The resulting oxygen deprivation stabilizes HIF-1α, which activates the transcription of pro-survival and pro-angiogenic genes such as vascular endothelial growth factor and key glycolytic enzymes while simultaneously impairing DNA repair mechanisms. (45-47)

These changes accelerate the emergence and selection of genetically unstable, aggressive cell clones. Hypoxia also acts in concert with TGF-β to amplify EMT, further enhancing the invasive potential of the affected epithelial cells. (45, 46)

5. Genetic Alterations and Mutations

Genetic instability is a hallmark of OSF progression to malignancy, with studies reporting cytogenetic damage in 47% to 53% of OSF samples. (38) Key genetic alterations include mutations in the TP53 tumor suppressor gene, which is one of the most common early events in OSF-derived OSCC. Mutant p53 protein loses its function as a "guardian of the genome," allowing cells with damaged DNA to proliferate unchecked. (48) Other significant genetic alterations include amplification of Cyclin D1 (a key regulator of the G1/S cell cycle transition), NOTCH1 inactivation (a key regulator of cell differentiation), and EGFR pathway activation (present in 80%-90% of head and neck squamous cell carcinomas). (49)

The areca nut components induce genetic damage through multiple mechanisms. Arecoline generates ROS that cause DNA strand breaks and base modifications (e.g., 8-hydroxydeoxyguanosine formation). (32, 42) Additionally, areca nut-specific nitrosamines formed during chewing have direct genotoxic effects, while the copper content in areca nuts activates LOX, which not only promotes collagen cross-linking but also contributes to oxidative DNA damage.(5, 35)

6. Epigenetic Modifications

Epigenetic reprogramming plays a crucial role in OSF malignant transformation, often preceding genetic alterations. The most extensively studied epigenetic change is promoter hypermethylation of tumor suppressor genes. Key genes frequently silenced in OSF and OSCC include p16INK4a (a cell cycle inhibitor), RASSF1A (involved in apoptosis and cell cycle arrest), DAPK1 (a pro-apoptotic gene), and E-cadherin (a key adhesion molecule whose loss is a hallmark of EMT). (46, 50, 51)

MicroRNA dysregulation is another important epigenetic mechanism in OSF transformation. Oncogenic miRNAs like miR-21 are significantly upregulated, while tumor-suppressive miRNAs like the miR-200 family (which maintains epithelial phenotype) are downregulated. (52) Similarly, long non-coding RNAs (lncRNAs) such as ADAMTS9-AS2 show decreased expression during malignant transformation. (53) These epigenetic changes collectively create a permissive environment for malignant transformation by altering the expression of critical genes without changing the DNA sequence itself.

TABLE 3: PATHOGENETIC FRAMEWORK OF OSF TO OSCC TRANSITION: MAJOR SIGNALING NETWORKS, GENETIC MUTATIONS, AND EPIGENETIC REPROGRAMMING

Mechanism / Pathway	Key Molecular Players	Main Effects in OSF→OSCC Progression
Oxidative stress and genotoxic damage	Reactive oxygen species (ROS), nitrosamines (from areca nut/tobacco)	DNA base modifications, strand breaks, lipid peroxidation, genomic instability
TGF-β signaling	TGF-β1, Smad2/3, lysyl oxidase (LOX, copper-dependent)	Early: suppresses epithelial proliferation. Chronic activation: fibroblast proliferation, myofibroblast differentiation, ECM deposition, collagen cross-linking, switch to tumor-promoting behavior
Epithelial–mesenchymal transition (EMT)	Snail, Slug, Twist, E-cadherin (↓), N-cadherin (↑),	Loss of cell–cell adhesion, acquisition of motility and

	vimentin (↑)	invasiveness, emergence of cancer stem-like traits
Hypoxia and metabolic adaptation	HIF-1α, VEGF, glycolytic enzymes	Angiogenesis, glycolytic shift, impaired DNA repair, selection of aggressive clones, cooperation with TGF-β to enhance EMT
Genetic alterations	TP53 mutations, Cyclin D1 amplification, NOTCH1 inactivation, EGFR activation	Loss of genome surveillance, uncontrolled G1/S transition, enhanced proliferation and survival
Epigenetic modifications	Promoter hypermethylation of p16INK4a, RASSF1A, DAPK1, E-cadherin; microRNAs (↑ miR-21, ↓ miR-200 family); lncRNAs (↓ ADAMTS9-AS2)	Silencing of tumor suppressor genes, disruption of apoptosis and cell-cycle control, facilitation of EMT and invasion

Role of Tumor Microenvironment and Immune Dysregulation

The tumor microenvironment (TME) in OSF undergoes significant changes that facilitate malignant transformation. The TME comprises various cellular components (fibroblasts, immune cells, endothelial cells) and acellular elements (cytokines, growth factors, extracellular matrix) that dynamically interact with epithelial cells to influence their behavior. (5, 6, 21)

- **Stromal Changes and Myofibroblast Activation:** A key feature of OSF is the activation of fibroblasts into myofibroblasts that are highly contractile, α-SMA-expressing cells that demonstrate increased proliferative capacity and excessive collagen production. (2, 38, 49) These activated myofibroblasts are the principal matrix-producing cells in OSF, generating massive amounts of ECM components including collagen types I and III, fibronectin, and proteoglycans . Importantly, myofibroblasts not only produce excessive ECM but also express tissue inhibitors of metalloproteinases (TIMPs), which suppress the activity of matrix-degrading enzymes (MMPs), creating an imbalance that favors matrix accumulation. (5, 17, 51) The persistent activation of myofibroblasts is maintained through autocrine and paracrine signaling involving growth factors like TGF-β, CTGF, and PDGF . Interestingly, some myofibroblasts in OSF may originate from epithelial cells through EMT, further contributing to the expanding pool of matrix-producing cells. (46, 49)
- **Immune Cell Infiltration and Cytokine Milieu:** The OSF microenvironment is characterized by chronic inflammation with infiltration of various immune cells, including lymphocytes, macrophages, and mast cells . There is a predominance of CD4+ T lymphocytes over CD8+ cells, and an increase in immunosuppressive cell populations such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). These immune cells release a plethora of pro-inflammatory cytokines (TNF-α, IL-1, IL-6) and profibrotic mediators (TGF-β, PDGF, CTGF) that drive both the fibrotic process and carcinogenesis. (45, 50, 51, 54, 55) TGF-β deserves special attention as a master regulator of fibrosis as it stimulates fibroblast proliferation, promotes their differentiation into myofibroblasts, enhances collagen synthesis, and inhibits collagen degradation through MMP suppression and TIMP induction. (45)
- **Micro-biome Dysbiosis and Chronic Inflammation:** Emerging evidence suggests that oral microbiome dysbiosis

plays a significant role in OSF pathogenesis and malignant transformation. Areca nut chewing alters the oral microbiome composition, reducing levels of commensal bacteria while increasing pathogenic species such as Streptococcus, Actinomyces, and Fusobacterium. (7, 10, 56) These pathogenic bacteria contribute to carcinogenesis through multiple mechanisms: they induce chronic inflammation through production of pro-inflammatory cytokines; generate carcinogenic metabolites; cause direct DNA damage; and modulate immune responses to favor immune evasion. (5, 6) Specifically, pathogens such as Porphyromonas gingivalis and Fusobacterium nucleatum have been implicated in OSCC progression through activation of oncogenic pathways and suppression of host immunity. (56)

Histopathological Progression

The transformation from OSF to OSCC follows a characteristic sequence of histopathological changes that correlate with clinical progression. In the earliest stages, the mucosa shows basal cell hyperplasia with hyperchromatism and the presence of a diffuse inflammatory infiltrate predominantly composed of lymphocytes, plasma cells, and eosinophils in the submucosa. Collagen fibers appear edematous and dispersed, with beginning signs of hyalinization. (57)

As the disease progresses to the intermediate stage, the inflammatory infiltrate becomes more pronounced and the collagen fibers undergo progressive hyalinization by losing their distinct fibrillar structure and forming homogeneous, eosinophilic masses. Fibroblasts demonstrate increased size and metabolic activity, with some transforming into myofibroblasts characterized by expression of α-SMA. (5, 47)

In advanced OSF, the histopathological picture is dominated by extensive hyalinization of the submucosal connective tissue, with thick, dense collagen bundles arranged in various orientations. The epithelium becomes markedly atrophic, often reduced to only a few cell layers thick, with loss of normal maturation patterns. Importantly, epithelial dysplasia characterized by cellular atypia, nuclear hyperchromatism, increased nuclear-cytoplasmic ratio, and loss of polarity develops in approximately 25% of OSF cases and represents a key step toward malignant transformation. (2, 46)

The final transition to invasive OSCC occurs when malignant epithelial cells breach the basement membrane and invade into the underlying fibrotic lamina propria and beyond. The dense collagen of OSF can initially act as a barrier to invasion, but eventually, cancer cells develop the proteolytic enzymes (e.g., MMPs) to degrade it and spread. (51, 53)

Biomarkers of Malignant Transformation

The identification of reliable biomarkers for predicting malignant transformation in OSF is crucial for early detection and intervention. Numerous molecules have been investigated as potential biomarkers, reflecting the various pathological processes involved in carcinogenesis. (44, 45, 48, 55)

- **Epigenetic Biomarkers:** DNA methylation markers show promise for early detection of malignant transformation. Promoter hypermethylation of tumor suppressor genes such as p16, RASSF1A, DAPK, and WIF-1 has been frequently observed in OSF tissues, with methylation rates increasing with disease progression. (49, 50, 54) Similarly, microRNA profiles have been identified in OSF, with increased expression of oncogenic miRNAs (e.g., miR-21) and decreased expression of tumor-suppressive miRNAs (e.g., miR-200b, miR-200c, miR-203). (52, 53)
- **EMT and Invasion-Related Biomarkers:** EMT markers provide valuable information about the transition to malignancy. Loss of E-cadherin expression and gain of N-cadherin and vimentin are characteristic changes observed during OSF transformation. Similarly, MMPs, particularly MMP9

and MMP12, show progressively increased expression from OSF to OSCC, reflecting the enhanced invasive capacity of transforming cells. (45, 46, 51, 54)

- **Proliferation and Apoptosis Markers:** Cell cycle regulators and apoptosis-related proteins demonstrate altered expression during malignant transformation. Increased expression of proliferation markers such as PCNA, Ki67, and cyclin D1 is observed in progressing lesions, while apoptosis inhibitors like survivin are upregulated and pro-apoptotic proteins like caspase-3 are downregulated. (58)
- **Oxidative Stress Markers:** Oxidative damage markers reflect the persistent oxidative stress in OSF tissues. 8-hydroxydeoxyguanosine (8-OHdG), a marker of DNA oxidation, is significantly elevated in OSF and OSCC tissues compared to normal mucosa. Similarly, nitric oxide (NO) and ceruloplasmin levels are increased, while antioxidant enzymes like superoxide dismutase (SOD) are depleted, indicating a state of chronic oxidative stress that promotes genetic instability. (1, 2, 5, 17, 44)

TABLE 4: MOLECULAR BIOMARKERS ASSOCIATED WITH THE PROGRESSION OF OSF AND ITS TRANSFORMATION TO OSCC

Mechanisms	Key Biomarkers	Findings in OSF/Transformation
Epigenetic	DNA methylation of p16, RASSF1A, DAPK, WIF-1	Promoter hypermethylation increases with disease progression, leading to silencing of tumor suppressor genes
	microRNAs (miR-21, miR-200b, miR-200c, miR-203)	Upregulation of oncogenic miR-21; downregulation of tumor-suppressive miR-200b/200c/203
EMT & Invasion	E-cadherin, N-cadherin, vimentin	Loss of E-cadherin with gain of N-cadherin and vimentin reflects epithelial–mesenchymal transition
	MMP9, MMP12	Expression progressively increases from OSF to OSCC, enhancing matrix degradation and invasion
Proliferation & Apoptosis	PCNA, Ki-67, cyclin D1	Elevated levels indicate increased cell proliferation
	Survivin, caspase-3	Survivin upregulated (anti-apoptotic); caspase-3 downregulated (pro-apoptotic)
Oxidative Stress	8-hydroxydeoxyguanosine (8-OHdG)	Significantly elevated, reflecting DNA oxidation
	Nitric oxide (NO), ceruloplasmin	Increased levels signal persistent oxidative stress
	Superoxide dismutase (SOD)	Depleted antioxidant enzyme, contributing to genetic instability

Clinical Management and Prevention Strategies

The management of OSF patients focuses on arresting disease progression and preventing malignant transformation. The cornerstone of management is complete cessation of areca nut and tobacco use, which may slow disease progression and reduce cancer risk, though established fibrosis is typically irreversible. (1, 53)

Early Detection and Surveillance

Regular monitoring of OSF patients is essential for early detection of malignant transformation. Clinical signs suggestive of transformation include the appearance of a non-healing ulcer, erythroplakia (red patch), verrucous or exophytic growth, rapid increase in trismus, or persistent pain. Biopsy of any suspicious area is mandatory for definitive diagnosis. (41)

Advanced diagnostic techniques may improve early detection capabilities. Liquid biopsy approaches analyzing saliva or blood for genetic and epigenetic alterations show promise for non-invasive monitoring. Optical diagnostic techniques such as tissue autofluorescence and toluidine blue staining can help identify high-risk lesions . DNA ploidy analysis has demonstrated high sensitivity and specificity for diagnosing epithelial dysplasia and early carcinoma in OSF patients, making it a valuable tool for cancer risk assessment. (17, 53)

Management

Antioxidant therapy (lycopene, beta-carotene, alpha-lipoic acid, vitamin E) represents a cornerstone of medical treatment, helping to counteract the oxidative stress that drives fibrogenesis and carcinogenesis . Immunomodulatory agents (corticosteroids, pentoxifylline, interferon-gamma) target the inflammatory component of OSF, while collagenase inhibitors (hyaluronidase) may help reduce fibrosis. (1, 44)

Surgical Intervention

For patients with advanced OSF and significant trismus, surgical intervention may be necessary to improve oral function. Various techniques have been employed including simple excision of fibrotic bands with primary closure or reconstruction using different flaps (buccal fat pad, tongue, palatal island, nasolabial, or radial forearm free flap). More recently, minimally invasive approaches such as laser fibrotomy have gained popularity, offering precise tissue ablation with reduced morbidity. (17, 44)

Regardless of the surgical technique used, postoperative physiotherapy is essential to maintain surgical results and prevent recurrence. Oral physiotherapy including active and passive mouth opening exercises, use of mechanical mouth openers, and heat therapy can help improve tissue elasticity and maintain mobility. (53)

Emerging Therapeutic Approaches

Novel targeted therapies are being developed based on improved understanding of OSF pathogenesis. These include monoclonal antibodies against profibrotic cytokines (anti-TGF-β, anti-CTGF), inhibitors of specific signaling pathways (JAK/STAT inhibitors, Wnt pathway inhibitors), and epigenetic modulators (DNA methyltransferase inhibitors, histone deacetylase inhibitors) . Gene therapy approaches aiming to restore balance between profibrotic and antifibrotic factors represent another innovative direction, though these remain largely experimental. (19, 44, 49, 53)

CONCLUSION

The transformation of OSF to OSCC represents a classic example of inflammation-driven cancer, where chronic exposure to areca nut components initiates a cascade of molecular events that progressively convert a fibrotic lesion into an invasive carcinoma. This process involves complex interactions between genetic susceptibility, epigenetic alterations, chronic inflammation, immune dysregulation, and microenvironmental changes that collectively drive carcinogenesis.

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