Oral submucous fibrosis - Current Concepts in Etiopathogenesis

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Abstract:
Oral submucous fibrosis (OSF) is now accepted globally as an Indian disease, having highest malignant potential than any other oral premalignant lesions. The understanding of the exact role of alkaloids and other etiological agents with respect to pathogenesis will help the management and minimize the blind clinical trials and treatment modalities. This article provides an overview of the etiopathogenesis with stress on the recent concepts related to this chronic “Indian Disease”.

Key Words: Areca nut, Arecoline, Cytokines, HLA typing, Oral precancer, Oral submucous fibrosis.

Introduction:
Oral submucous fibrosis (OSF), now globally accepted as an Indian disease, has one of the highest rates of malignant transformation amongst potentially malignant oral lesions and conditions. The condition has also been described as idiopathic scleroderma of mouth, idiopathic palatal fibrosis and sclerosing stomatitis. It was first described three decades ago by Pindborg & Sirsat (1966). The hallmark of the disease is submucosal fibrosis that affects most parts of the oral cavity, pharynx and upper third of the esophagus leading to dysphagia and progressive trismus due to rigid lips and cheeks.

The disease is predominantly seen in Asian countries, prevalence being more in India (Fig. 1).

Recent epidemiological data indicates that the number of cases of OSF has risen rapidly in India from an estimated 250,000 cases in 1980 to 2 million cases in 1993. The reasons for the rapid increase of the disease are reported to be due to an upsurge in the popularity of commercially prepared areca nut preparations (pan masala) in India (Ranganathan et al, 2004) and an increased uptake of this habit by young people (Gupta et al, 1998) due to easy access, effective price changes and marketing strategies.

Oral Submucous fibrosis is diagnosed on the basis of clinical criteria including oral ulceration, paleness of the oral mucosa and burning sensation (particularly in the presence of spicy foods), hardening of the tissue and presence of characteristic fibrous bands. The fibrosis involves the lamina propria and the submucosa and may often extend into the underlying musculature resulting in the deposition of dense fibrous bands giving rise to the limited mouth opening which is a hallmark of this disorder.

Etiology:
Epidemiological data and intervention studies suggest that areca nut is the main aetiological factor for OSF (Pindborg et al, 1984; Seedat & Van Wyk, 1988; Sinor et al, 1990; Maher et al, 1994; Murti et al 1995; Merchant et al, 1997; Shah & Sharma, 1998; Yang et al, 2001; Farrand et al, 2001; IARC, 2004; Jacob et al, 2004). Other etiological factors suggested are chillies, lime, tobacco, nutritional deficiencies such as iron and zinc, immunological disorders, and collagen disorders. Extensive research is being done on the Indian supari (areca nut / betel nut, Fig. 2).
Fig. II: Areca nut – Indian supari is the main cause for submucous fibrosis

Areca nut:

The term areca nut is used to denote the unhusked whole fruit of the areca nut tree and term betel nut is used exclusively to refer to the inner kernel or seed which is obtained after removing husk. The betel nut has psychotropic and anti helminthic property due to presence of areca alkaloids. Four alkaloids have been conclusively identified in biochemical studies, arecoline, arecaidine, guvacine & guvacoline, of which arecoline is the main agent. These alkaloids have powerful parasympathetic properties which produce euphoria and counteract fatigue.

Nitrosation of arecoline leads to the formation of areca nut specific nitrosamine namely nitrosoguvacoline, nitrosoguvaicine and 3-methyl nitrosominopropionitrile, which alkylate DNA. Metabolism of these areca nut specific nitrosamine lead to formation of cyanoethyl, which binds with o’methyl guanine in DNA. Prolonged exposure to this irritant leads to malignant transformation.

Recently suggested pathogenesis of oral submucous fibrosis is by dual action of areca nut. It is suggested that arecoline not only stimulates fibroblastic proliferation and collagen synthesis but also decreases it’s breakdown. This suggests that arecoline is the active metabolite in fibroblast stimulation.

Areca and slaked lime:

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

Tobacco & Lime:

These are known irritants and causative factors in oral malignancy. They may act as local irritants. The commercially freeze dried products such as Pan masala, Gutka and Mawa (areca, tobacco and lime) have high concentrates of areca nut per chew and appear to cause OSF more rapidly than by self prepared conventional betel quid which contain smaller amounts of areca nut. (Shah & Sharma, 1998; Sinor et al, 1990).

Chillies:

The use of chillies (Capsicum annum and Capsicum frutescense) has been thought to play an etiological role in oral submucous fibrosis. Capsaicin, which is vanillylamide of 8-methyl-6-nonenic acid, is the active ingredient of chillies, play an etiological role in oral submucous fibrosis (Rajendran, 1994).

Nutritional deficiency:

A subclinical vitamin B complex deficiency has been suspected in cases of OSF with vesiculations and ulcerations of oral cavity. The deficiency could be precipitated by the effect of defective nutrition due to impaired food intake in advanced cases and may be the effect, rather than the cause of the disease (Rajendran, 1994).

Defective iron metabolism:

Microcytic hypochromic anemia with high serum iron has been reported in submucous fibrosis (Rajendran, 1994).

Collagen disorders:

Oral submucous fibrosis is thought to be a localized collagen disease of oral cavity. It is linked to scleroderma, rheumatoid arthritis, Duputreyen’s contracture and intestinal fibrosis. A link between scleroderma and oral submucous fibrosis has also been suspected on the basis of similarity of histological characteristics (Tsai et al, 1999; Tilakaratne et al, 2005).
**Immunological disorders:**

Raised ESR and globulin levels are indicative of immunological disorders. Serum immunoglobulin levels of IgA, IgG and IgM are raised significantly in oral submucous fibrosis. These raised levels suggest an antigenic stimulus in the absence of any infection. Circulating autoantibodies are also present in some cases of oral submucous fibrosis (Canniff et al, 1985).

**Pathogenesis:**

**I Collagen accumulation:**

Oral submucous fibrosis results from increased production of collagen by fibroblasts. In addition to this there is decreased breakdown leading to accumulation of excessive amount of collagen.

a) Increased Collagen Production:

Under the influence of areca nut, fibroblasts differentiated into phenotypes that produce more collagen. The alkaloids present in areca nut, arecadin and arecoline are responsible for this. Arecoline gets converted in to arecadine which is the active metabolite. There is dose dependent increase in production of collagen by fibroblasts under influence of these factors (Flow chart-1; Meghji et al, 1987; Kuo et al, 1995).

Various cytokines are increased in oral submucous fibrosis. These are: Transforming growth factor (TGF-β), Platelet derived growth factor (PDFG) and Basic fibroblast growth factor (bFGF). These are fibrogenic growth factors that stimulate collagen production. Another cytokine that has anticollagen effect is Interfran-α (IFN-α). This is decreased in OSF. Thus overall there is stimulation of collagen synthesis through different mechanisms (Haque et al, 1998).

b) Stabilization of collagen structure and decreased collagen breakdown:

One of the mechanisms that can lead to increased fibrosis is by reduced degradation of collagen by forming a more stable collagen structure. Betel nut contains tannin. Tannin has ability to stabilize collagen by cross-linking it. With the progression of the disease type III collagen is almost completely replaced by type I (Utsunomiya et al, 2005). Type I collagen is more resistant to degradation than type III. An important finding from these studies is the identification of excess α-1 chains relative to α-2, suggesting an alteration of collagen molecules during the progression of the disease. Although, the biological function of this trimer is not known, it is regarded as more resistant to degradation than the normal collagen molecule (Tsai et al, 1999).

Another component of betel nut that aids this cross-linking is copper. It is a constituent of enzyme lysyl oxidase. This enzyme also causes cross-linking and makes collagen resistant to degradation by collagenase. Due to action of tannin and copper, collagen that is produced in OSF is highly resistant to remodeling and phagocytosis (Tsai et al, 1999).

It is fibroblast that brings about remodeling and phagocytosis of collagen. As in OSF, these fibroblasts are affected and phenotypically changed, they cannot degrade collagen. Studies on the effects of arecoline on both normal and OSF fibroblasts in culture revealed an elevated rate of collagen synthesis by OSF fibroblasts as compared to normal fibroblasts.

![Flow chart 1: Role of areca alkaloids in OSF (Ghom & Mhaske, 2008).](image1)

![Flow chart 2: Role of areca nut in oral submucous fibrosis (Ghom & Mhaske, 2008).](image2)
Although the reason for this elevation is not clear, some authors proposed that it could reflect the clonal selection of a highly fibrogenic cell population in the altered tissue under the influence of local factors such as interleukin-1 from inflammatory cells (Utsunomiya et al, 2005). This leads to decrease in phagocytosis & accumulation of collagen in oral mucosa (Flow chart 2). Glycogen consumption is physiologically related to cellular activity of muscle fibres. Over activity of muscles results in excessive glycogen consumption, leading to its depletion. In areca nut chewers there is overactivity of muscles due to repeated, continuous chewing and use of heavy force to crush the hard nut. This increased muscle activity along with diminished blood supply, following connective tissue changes leads to muscle degeneration and fibrosis.

**II Increased expression of fibrogenic cytokines:**

The most important finding in the various studies was the demonstration of increased expression of fibrogenic cytokines namely TGF β-1, PDGF and bFGF in OSF tissues compared to normal (Haque et al, 1998). These observations may suggest that the disease process in OSF may be an altered version of wound healing as recent findings show that the expression of various ECM molecules are similar to those seen in maturation of granulation tissue. (Utsunomiya et al, 2005)

**III Genetic polymorphisms predisposing to OSF:**

Polymorphisms of the genes coding for TNF-α has been reported as a significant risk factor for OSF. TNF-α is known to stimulate fibroblastic proliferation in vitro (Vilcek et al, 1986). Evidences suggest that collagen-related genes are altered due to ingredients in the quid. The genes COL1A2, COL3A1, COL6A1, COL6A3 and COL7A1 have been identified as definite TGF-β targets and induced in fibroblasts at early stages of the disease. The transcriptional activation of these procollagen genes by TGF-β suggests that it may contribute to increased collagen levels in OSF (Rajalalitha & Vali, 2005).

**IV OSF as an autoimmune disorder:**

OSF is also thought to be an autoimmune disease. The presence of various autoantibodies in varying titers is reported in several studies confirming autoimmune basis to the disease. Few studies have reported on HLA typing of OSF patients and controls. Higher frequencies of OSF are found in HLA A10, DR3 and DR7 types when compared to an ethnically, regionally and age-matched control group (Cannif et al, 1985; Kuo et al, 1995). Although the data on various HLA types, raised autoantibodies and the detection of immune complexes tend to indicate an autoimmune basis for the disease, substantial number of cases and matched controls may be required to verify these findings (Tilakaratne et al, 2005)

**Precancerous nature and malignant transformation:**

The precancerous nature of OSF was first described by Paymaster in 1956 when he observed slow growing squamous cell carcinoma (SCC) in one third of the patients with the disease. This was confirmed by various other workers. Pindborg in 1972 put forward five criterias to prove that the disease is precancerous (Pindborg et al, 1984). They included, high occurrence of OSF in oral cancer patients, higher incidence of SCC in patients with OSF, histological diagnosis of cancer without any clinical suspicion in OSF, high frequency of epithelial dysplasia and higher prevalence of leukoplakia among OSF cases.

Most of the earlier studies have focused on the prevalence of epithelial dysplasia in OSF. It has so far been the most reliable indicator for predicting potential malignant transformation of an oral precancerous lesion though new markers are emerging (Warnakulasuriya, 2001). Epithelial dysplasia in OSF tissues appeared to vary from 7 to 26% depending on the study population (Pindborg et al, 1967; Murti et al, 1995; Lee et al, 2003). However, according to the current awareness of the disease and some refined criteria for grading dysplasia, it is reasonable to assume that the prevalence of dysplasia is more towards the midway of the reported range. Malignant transformation rate of OSF was found to be in the range of 7–13%.

The hypothesis that dense fibrosis and less vascularity of the corium, in the presence of an altered cytokine activity creates a unique environment for carcinogens from both tobacco and areca nut to act on the epithelium is widely being accepted. It could be assumed that carcinogens from areca nut accumulate over a long period of time either on or immediately below the epithelium allowing the carcinogens to act for a longer duration before it diffuses into deeper tissues.
Conclusions:
Various available data suggests that the main causative agents for OSF are the constituents of areca nut, mainly arecoline, whilst tannin may have a synergistic role. Arecoline will interfere with the molecular processes of deposition and/or degradation of extracellular matrix molecules such as collagen. Due to this interference, phagocytic capacity of fibroblast is reduced, because of up or down regulation of key enzymes such as lysyl oxidase and alteration in expression of various ECM molecules. The process may also be influenced by increased secretion of inflammatory cytokines, growth factors and decreased production of anti-fibrotic cytokines. Although the above mechanisms may explain the induction, maintenance and progression of fibrosis in OSF, further research is required in order to identify the mechanisms leading to carcinogenesis in this fibrotic oral mucosa. Nutritional deficiencies may not play a primary role but it could synergize the symptomatology by contributing to epithelial atrophy. Although the involvement of HLA and genetic predisposition has been reported, specific haplotypes have not been determined. The individual mechanisms operating at various stages of the disease–initial, intermediate and advanced–need further study in order to propose appropriate therapeutic interventions.

Bibliography:

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