Etiopathogenesis of oral submucous fibrosis

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Abstract

Etiopathogenesis of oral submucous fibrosis (OSF) is been studied for long time and still a fully satisfactory explanation is not obtained. Areca nut is the main etiological factor which is consumed as pan masala, gutka, mawa, and betel quid. It will interfere with the collagen synthesis and degradation causing the fibrosis. Several other etiological agents such as chilli and nutritional deficiency have been studied. Furthermore, the genetic susceptibility and autoimmune nature of disease have been postulated by some others. Current evidence also implicates collagen-related genes in the susceptibility and pathogenesis of OSF.

Keywords:
Arecadine, arecanut, arecoline, chilli, oral submucous fibrosis, tissue growth factor-β

Introduction

Oral submucous fibrosis (OSF) is an oral disease, which was first described by Schwartz. It is a potentially malignant condition. This leads to a restricted mouth opening, resulting in trismus leading to restriction of food consumption, difficulty in maintaining oral health, as well as impairs the ability to speak. The pathogenesis of the disease is believed to be multifactorial. Numerous factors trigger the disease process by causing juxta epithelial inflammatory reaction in the oral mucous membrane. The most common factors include areca nut chewing, ingestion of chilies, genetic and immunologic processes, nutritional deficiencies, and other factors among which betel quid (BQ) chewing has been recognized as the most important risk factor.

Areca Nut

A number of epidemiological studies provide overwhelming evidence that the areca nut is the main etiological factor for OSF. Most convincing evidence is derived from case-control studies that estimate the odds ratios for areca nut use among OSF cases and a definite dose-dependent relationship between areca nut and causation of the disease. Daily use appeared to be more important than the duration of the habit. A hospital-based case-control study on habits and OSF was carried out by Ranganathan et al. in 2004 which revealed a male to female ratio of 9.9:1. Areca nut in all forms is associated with OSF, with the risk being the highest with pan masala. The habit duration was more significant than the frequency of the chewing habit. Risk of developing OSF was almost double for subjects below 21 years of age compared with that for the 21-40 year age group; the younger features of OSF in 3.5 years whilst the older group took 6.5 years from the start of the habit. In a case-control study by Jacob et al. in Kerala, India, conferred odds ratio of 56.2 for OSF among nonsmokers and nondrinkers, chewing BQ without tobacco, response relationships were observed for both the frequency and duration of BQ chewing without tobacco on the risk of oral precancers. OSF cases chewed BQ without tobacco at a higher frequency relative to the other oral precancer cases. Shah and Sharma studied 236 consecutive cases of OSF and found that chewing of quid/areca nut or pan masala was directly associated with OSF. Furthermore, pan masala (a commercial preparation of areca nuts, lime, catechu and undisclosed coloring, flavoring, and sweetening agents) was consumed by a relatively younger age group and was associated with OSF changes earlier than areca nut/quid chewing. However, chewing or smoking tobacco with many other chewing habits did not increases the risk of developing OSF. It was also found that frequency rather than the duration of the chewing habit was directly associated to OSF.

As areca nut consists of tannins, of which gallotannic acid and D-catechol are most important. The major alkaloids are arecoline, arecaidine, arecoline, guyacoline, isoguvacine, and...
guvacine. The important flavonoid components of areca nut are tannins and catechins.\[6\] Arecoline is the most abundant alkaloid. These alkaloids undergo nitrosation to form N-nitrosamines, which might have a cytotoxic effect on cells. Arecoline has been demonstrated to promote collagen synthesis. The alkaloids and flavonoids from the BQ are absorbed and undergoes metabolism. These constituents and their metabolites are a source of constant irritation to oral tissues. In addition to the chemical irritation from BQ constituents and their metabolites, the coarse fibers of areca nut also cause mechanical irritation to the oral mucosa. Furthermore, the microtrauma caused due to the friction of coarse fibers of areca nut also leads to the diffusion of BQ alkaldoids and flavonoids into the subepithelial connective tissue, which results in juxta epithelial inflammatory cell infiltration. Any external factor, which causes any form of injury to tissue, elicits a protective inflammatory process. Over a period of time, persistent habit leads to chronic inflammation at the site. Initial irritation leads to further atrophy and ulceration of the mucosa. There is an elaboration of various chemical mediators of inflammation, especially prostaglandins (PGs) plays an essential role. PGs secretion by oral keratinocytes in response to areca nut extract has been shown. It has been shown that areca nut extracts can lead to PGs secretion by oral keratinocytes. Aberrant and persistent tissue inflammation is crucial for the occurrence of cancer and tissue fibrosis. Thus, it can be considered that induction of inflammation of oral mucosa by BQ ingredients to be a critical event in the pathogenesis of OSF. Cytokines like interleukin 6, tumor necrosis factor interferon alpha, etc. and growth factors like tissue growth factor-alpha (TGF-α) are synthesized at the site of inflammation. Increased susceptibility among individuals who are anemic due to iron or vitamin B12 deficiencies has been demonstrated. This could be due to increased fragility of the mucosa by which there is more BQ absorption. TGF-α 1 is a key regulator of extracellular matrix (ECM) assembly and remodeling. The action of TGF-α on the genes implicated in the formation and degradation of the ECM is mostly exerted at the transcriptional level through ill-defined intracellular pathways. The molecular events can be seen in two main sections: collagen production pathway and collagen degradation pathway, as regulated by TGF-α and the flavonoids present in areca nut.\[6\]

The three main events which are mediated by TGF-α, which favors the collagen production, are:  
1. Activation of procollagen genes  
2. Elevation of procollagen proteinases levels: (a) Procollagen C-proteinase (PCP)/bone morphogenetic protein1 (BMP1) and (b) procollagen N-proteinase (PNP)  
3. Up-regulation of lysyl oxidase (LOX) activity.

Elevation of procollagen proteinases levels. Procollagen proteinases play an essential role in processing. There are two types of proteinases that cleave the N- and C-terminal respectively - PNP and PCP.\[6\]

PCP - The PCP and BMP1 have been shown to be the same protein that cleaves the C-terminal of procollagen precursor. TGF-α has been found to induce BMP1 at the transcriptional and translational levels in different cell types such as the osteosarcoma cells and fibrogenic cell cultures.\[6\]

PNP - It cleaves the N-propeptide of procollagen precursor. There are two types of PNP, PNP I and III, they are classified based on the type of procollagen fibers on which they act. TGF-beta (TGF-β) treated cells have been shown to have an elevated level of PNP. Thus, not only is procollagen gene expression increased by TGF-α, but also their processing into fibrils is enhanced by increased levels and activities of the PNP and PCP.\[6\]

Up-regulation of LOX, the LOX is an enzyme which finally process collagen fibers into a stabilized covalently cross-linked proteolysis resistant mature fibrillar form. The LOX is dependent on copper for its functional activity. During the biosynthesis of LOX, copper is incorporated into LOX. Apart from copper, LOX also contains another co-factor, a covalently bound carbonyl prosthetic group - lysine tyrosyl quinone (LTQ). The LTQ is essential for the reaction mechanism of LOX, i.e., in the formation of cross-links in the collagen fibers. Copper has been suggested to play a structural role in stabilizing the LTQ. During the process of cross-linking, copper plays an important role in reoxidizing the reduced enzyme facilitating the completion of the catalytic cycle. Areca nuts have a high copper content, and areca nuts consumption for 5-30 min causes significant increase in soluble copper levels in oral fluids which could act as an important factor in OSF by fibrogenesis through up-regulation of LOX activity. Apart from this, the flavonoids that are present in areca nut have been implicated in enhancing the cross-linking of collagen fibers. In vitro studies have demonstrated the presence of catechin to raise LOX activity. They might be oxidatively converted to quinones and hence, might resemble LTQ, which is an important co-factor for LOX activity TGF-α has been found to strongly promote the expression of LOX both at the mRNA and protein levels in various cell lines. This could be indirectly via the elevation of BMP1 by TGF-α as it mediates the biosynthetic processing of LOX. The studies have shown that the daily intake of copper in American diets averages about 1-0 mg about 60% of which is absorbed. In a study it was found that an adult Indian chewing areca daily will consume over 5 mg of copper of which a substantial but unknown quantity will be absorbed, particularly in those who swallow substantial amounts of quid juice.\[7\]

Collagen degradation pathway. There are two main events modulated by TGF-α, which decreases the collagen degradation:\[8\]

(i) Activation of tissue inhibitor of matrix metalloproteinase gene (TIMPs)  
(ii) Activation of plasminogen (PLG) activator inhibitor (PAI) gene.  

Activation of TIMP gene: Matrix metalloproteinases (MMPs) constitutes a set of structurally related matrix degrading proteases. They are endopeptidases that play an essential role in tissue remodeling by degrading ECM, both in health and disease. The MMPs are of many types, but MMP1, MMP8, and MMP13 are referred to as collagenases. The flavonoids have been shown to
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Chilli

OSF is seen mostly among population groups who use chillies (*Capsicum annum* and *Capsicum frutescense*). During the initial phase of the disease itself OSF patients are unable to tolerate spicy food containing chillies. Sirsat and Khanolkar[9] observed epithelial hyperplasia, the presence of a chronic inflammatory cell exudate, and dense fibrogenesis with elastotic degeneration of collagen; similar to the chronic productive response in OSF specimens.

Experimentally capsaicin had evoked enhanced connective tissue response enhanced in protein- or vitamin-deficient animal systems. However, many investigators failed to produce the same result with chilli.[14-16] Pindborg and Singh[15-17] suggested an allergic nature of this disease possibly due to chilli intake. The studies by Seedat and van Wyk[18] showed no positive correlation.[9]

Chilli hypothesis do not hold in places like Mexico and South America where chilli is widely used and OSF is not found. The overall assessment is that there is no evidence substantiating the etiologic role of chillies in OSF.[20]

Recent experiments have shown chilli extract to be mutagenic[20] and can enhance the tumorigenicity of tobacco in experimental animals.[21] Furthermore, epidemiologic studies have shown that chilli consumption increases the risk of cancers in the upper aero digestive tract in a dose-dependent manner.[22]

Role of Minerals

Hemoglobin and serum iron is found to be reduced in OSF.[23-25] OSF is basically a collagen disorder. Hydroxyproline is an amino acid found only in collagen and is incorporated in collagen with help of iron and ascorbic acid. The decrease in iron levels may be due to utilization of iron in fibrosis.[26] Lack of iron causes improper vascular channel formation and decrease in vascularity. It causes defective healing and scarification due to altered inflammatory response in lamina propria. Furthermore, it leads to decrease in levels of cytochrome (CYP) oxidase which causes epithelial atrophy and make mucosa vulnerable to irritants.[27]

In OSF cases there will be decreased serum zinc. Serum and salivary copper levels are elevated. This will up regulate LOX leading to fibrosis. Copper increases the half-life of LOX and lead to more cross-linking of collagen and elastin.[27,28]

Various other trace elements have been evaluated in OSF, including K, Si, Ca, V, Cr, Ni, Mn, Br, Rb, Sr, Co, and Pb. In an analysis on 16 trace elements in OSF, gross depletion of Zn, Br, and Fe was found while Mn and Co showed an increase in blood concentrations.[29]

The deficiency status of minerals may be secondary to the inability to open mouth or intolerance to spicy food.

Role of Genetic and Immunologic Factors

Canniff et al. performed human leukocyte antigen (HLA) tissue typing and observed that the frequencies of HLA A10, DR3, and DR7 were significant.[30] This observation imply a possible genetic susceptibility to the action of extraneous factors such as areca alkaloids and tannins. The familial occurrence of this condition has also been reported.[31,32] The occurrence of OSF among individuals without areca nut chewing habits has also been thought to be due to genetic factors.[33]

The genes COL1A2, COL3A1, COL6A1, and COL6A3 were found to be related to progression of the disease. Furthermore, polymorphism of gene coding for TNF-β has been reported in OSF which in turn inhibits collagen phagocytosis.[34]

Single nucleotide polymorphisms in Thr 241 Met - NAT2 A857G increase the risk of OSF.[35] Connective TGF (CTGF/CCN2) is also increasingly expressed in OSF. This may be due to upregulation of CCN2 by thrombin produced by microtrauma.[36]

Glutathione S-transferases (GSTs) GSTM1 and GSTT1 is also found to be higher in the OSF.[37]

Areca nut chewing and alcohol abuse cause increased MMP1 Single nucleotide polymorphisms.[38] Polymorphism in the MMP3 promoter can increase susceptibility to develop OSF.

Arecoline can elevate heme oxygenase-1 expression in dose dependent manner especially in fibroblasts, endothelial cells, and inflammatory cells.[39]
Other gene changes demonstrated in OSF were cartilage oligomeric matrix protein, CYP 3A5, antinuclear, antismooth muscle, antigastric parietal cell, antithyroid microsomal, antireticulin antibodies, endothelin-1, and vimentin.

Immunohistochemistry showed predominance of T-lymphocytes and macrophages in epithelium and subepithelial connective tissue with CD4+ to CD8+ lymphocyte ratio of 2.1:1. Plasma Fibrin degradation products were also increased in connective tissue which cause excessive deposition of fibrin leading to restriction of mouth opening.

Autoimmunity
Suspicion of an autoimmune explanation for OSF stems from certain similarities of this condition with other collagen disorders, namely scleroderma, which is presumed to have an autoimmune pathogenesis. Because of the similarities of clinical features between OSF and scleroderma, earlier it was thought as oral scleroderma. The ultrastructural changes in this condition were also similar to those seen in rheumatoid arthritis and scleroderma. It became apparent that DR antigen, which indicates an autoimmune basis of a disease, was associated with scleroderma. In view of the female bias, the age of onset of this condition, and other immunologic and genetic findings, the authors felt that OSF, like scleroderma, could have an autoimmune basis.

Cytotoxic T-lymphocyte antigen 4 pleomorphism seen in OSF is also observed in autoimmune diseases such as systemic lupus erythematosus, insulin-dependent diabetes mellitus, Graves’ disease, Hashimoto thyroiditis, multiple sclerosis and rheumatoid arthritis which by some authors point to autoimmune origin.

Role of Infection
An important link between OSF and decreased immune response may be the suggested viral origin of the disease. Human papillomavirus DNA, herpes simplex virus DNA and Epstein–Barr virus DNA were detected from some patients with OSF. Viral lesions show similar immune derangements such as abnormal CD4/CD8 cell ratio as seen in OSF. Furthermore, viral antigen can elicit specific suppressor T-cell response. The resulting immunosuppression allows the spread of viral antigen and associated transformation of epithelium. A defect in target cell or viral cell lysis is seen by natural killer NK cells in OSF.

Others
Serum beta carotene level was found to be inversely proportional to the disease progression in the patients with OSF. Maximum reduction was evident for Grade III OSF.

Summary
Flowchart 1 depicts the summary of current understanding on etiopathogenesis of OSF.

Flowchart 1: Summary of current understanding on etiopathogenesis of oral submucous fibrosis
Conclusion

A long span of time has passed since the first diagnosis of OSF, and various treatment modalities have been tried for the management of same. However, the success rate for the treatment of OSF has been relatively low. The reasons for this may be attributed to unknown etiopathogenesis of this disease. After having a glance at the vast literature of OSF, it can be said that there is a further scope for research to elicit the etiopathogenesis and subsequent management of this condition, so that we can provide a better standard of living to our patients suffering from this potentially malignant condition.

References
