Review Article

ORAL SUBMUCOUS FIBROSIS: AN UPDATE ON ETIOLOGY AND PATHOGENESIS-A REVIEW

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Abstract: Oral submucous fibrosis (OSMF) is a chronic disease of oral mucosa characterized by inflammation and progressive fibrosis followed by stiffening of an otherwise yielding mucosa resulting in difficulty in opening the mouth. It is generally accepted today that areca nut quid plays a major role in the etiology. The pathogenesis of the disease is not well established, but the cause of OSMF is believed to be multifactorial. Ingestion of chillies, genetic susceptibility, nutritional deficiencies, and autoimmunity and collagen disorders may be involved in the pathogenesis of this condition. The purpose of this review is to analyze critically the recent developments that may lead to understanding of etiology of OSMF. The review article has been prepared doing a literature review from the World Wide Web and pubmed/medline.

Keywords: Oral; Fibrosis; OSMF; Autoimmunity; Pathogenesis; Arecanut.

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic disease of oral mucosa characterized by inflammation and progressive fibrosis of lamina propria and deeper connective tissues, followed by stiffening of an otherwise yielding mucosa resulting in difficulty in opening the mouth.[1,2]Oral sub-mucous fibrosis is a chronic condition of the oral mucosa, first describe among five East African women of Indian origin under the term, atrophia idiopathica (tropica) mucosae oris. Since then this condition has also been described as, idiopathic scleroderma of the mouth, idiopathic palatal fibrosis, Sclerosing stomatitis.In India this condition was first describe as diffuse Oral sub-mucous fibrosis and as sub-mucous fibrosis of the palate and pillars.[3]

Several other descriptive terms have been attributed; sub mucosal fibrosis of palate and pillars, diffuse oral submucous fibrosis, idiopathic scleroderma of the mouth, idiopathic palatal fibrosis, and sclerosing stomatitis.[4]The possible precancerous nature of sub mucous fibrosis was first mentioned by Paymaster in 1956, who described the development of slow-growing squamous cell carcinoma in one third of the cases with sub mucous fibrosis.[5]The etiology of the disease over the intervening years was thought to be multifactorial and several agents have been implicated, including the consumption of large amounts of chillies, nutritional deficiency, genetic predisposition, and auto-immune disease. Conclusive evidence now exists indicating that OSF is caused by areca nut, a masticatory substance used predominantly by peoples of South and SE Asian ethnicity, the surrounding geographical areas, and in the diaspora there from.

Several pathogenic mechanisms have been proposed, all based on the constituents of areca nut and genetic susceptibility to the disease. In essence, the disease could be described as primarily as a collagen metabolic disorder with changes observed in the extracellular matrix of the lamina propria and in the deeper mucosal tissues of the oral cavity because of both increased collagen synthesis and /or reduced collagen degradation. Epithelial changes are more likely to be secondary events. This article highlights an update on the etiology and pathogenesis of oral submucous fibrosis.[4]

AETIOLOGY

Previous studies on the pathogenesis of OSF have suggested that the occurrence may be due to:

(a) Clonal selection of fibroblasts with a high amount of collagen production during the long-term exposure to areca quid ingredients.

(b) Stimulation of fibroblast proliferation and collagen synthesis by areca nut alkaloids.

(c) By fibrogenic cytokines secreted by activated macrophages and T lymphocytes in the OSF tissue.
(d) By decreased secretion of collagenase.
(e) Deficiency in collagen phagocytosis by OSF fibroblasts.
(f) By production of collagen with a more stable structure (collagen type I trimer) by OSF fibroblasts.
(g) By stabilization of collagen structure by (+) catechin and tannins from the areca nut.[6]

A number of epidemiological surveys, case-series reports, large sized cross sectional surveys, case-control studies, cohort and intervention studies provide over whelming evidence that areca nut is the main aetiological factor for OSF. Most convincing evidence is derived from case-control studies that estimate the odds ratios for areca nut use among oral submucous fibrosis cases and a definite dose-dependent relationship between areca nut and causation of the disease. There are numerous biological pathways involved in the above processes and, it is likely that the normal regulatory mechanisms are either down regulated or up regulated at different stages of the disease.[7,8,9,10,11,12]

ARECA ALKLOIDS

Chemical constituents and alkaloids from arecanut have the most important role in pathogenesis of oral submucous fibrosis. Harvey M investigated the metabolism of arecoline, the major alkaloid in the nut, by human buccal mucosa fibroblasts in vitro. Alkaloid metabolites extracted from culture media were analysed by gas chromatography and thin-layer chromatography. [3H]-arecoline was metabolized predominantly to [3H]-arecaidine and this was accompanied by a concentration-dependent stimulation of collagen synthesis and cell proliferation. Arecaidine was a more potent stimulator than arecoline. The rate of hydrolysis of a series of synthetic arecaidine esters (methyl, ethyl, butyl, propyl and pentyl) by fibroblasts was closely correlated with the extent of stimulation of collagen synthesis. Author concluded that fibroblasts are responsive to the major metabolite of arecoline and hydrolysis of the ester group may be necessary for this action. Following four alkaloids have been conclusively identified in biochemical studies:

- Arecoline
- Arecaidine
- Guvacine
- Guvacoline

Out of these constituents arecoline is the main agent. Hydrolysis of arecoline produces arecaidine that has pronounced effects on fibroblasts. It was suggested that arecaidine is the active metabolite in fibroblast stimulation.(Figure:1) This hypothesis 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 making this agent available in the oralenvironment.[13]

Figure 1: Role of areca alkaloids in oral submucous fibrosis[13]

Another study reports that arecoline influences deposition of extracellular matrix (ECM) by increasing the production of TIMP-1. The effect is enhanced when fibroblasts are co cultured with keratinocytes, suggesting that interaction of oral keratinocytes and fibroblasts play an important role in the pathogenesis of OSF. In addition, the interaction of arecoline and keratinocytes is reported to induce the differentiation of myofibroblasts from fibroblasts. A different study suggests that areca alkaloids induce buccal mucosal fibroblast contraction and this process was related to phospholipase C, inositoltriphosphate 3, Ca^{2+}, calmodulin and

Rho signaling pathway as well as act in filament polymerization. The persistent fibroblast contraction may induce fibrotic process in OSF.[14,15,16]

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 micro trauma produced by the friction of coarse fibers of areca nut also facilitates the diffusion of BQ alkaloids and flavonoids into the subepithelial connective tissue, resulting in juxta epithelial inflammatory cell infiltration.

Any external factor, which causes any form of injury to tissue, can elicit a protective inflammatory process. Over a period of time, due to persistent habit, chronic inflammation sets in at the site. (Figure:2) Initial irritation leads to further atrophy and ulceration of themucosa.[17]

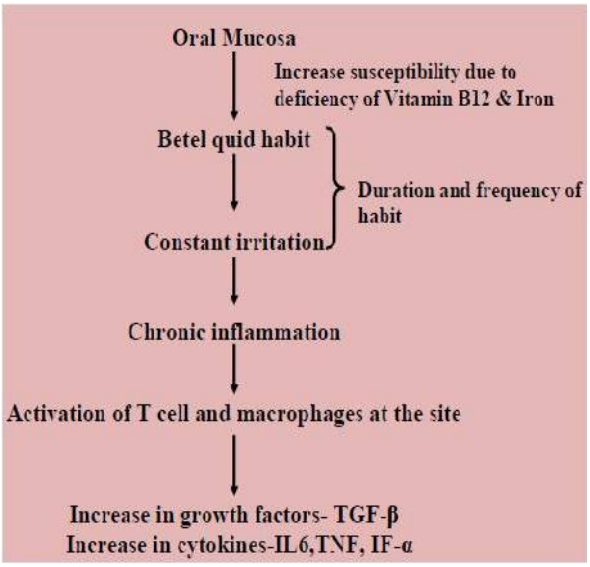

Figure 2: Initial events in progression of disease[17]

COLLAGEN PRODUCTION PATHWAY [18,19,20]

The three main events that are modulated by TGF-β, which favours the collagen production, are:

(1) Activation of procollagen genes.

(2) Elevation of procollagen proteinases levels:

(a) Procollagen C-proteinase (PCP)/bone morphogenetic protein1 (BMP1)

(b) ProcollagenN-proteinase (PNP)

(3) Up-regulation of lysyloxidase (LOX) activity.

1. **ACTIVATION OF PROCOLLAGEN GENES**

Collagen plays a critical role as a structural element of connective tissue. About 27 types of collagen have been recognized, which can be grouped into seven broad classes. Major class is fibrillar collagen, among them types I, III, and VI form a major part of connective tissue. Collagen type VII forms the anchoring fibrils of oral mucosa. The distinguishing feature is a unique type of triple helix, stabilized by unusual cross-links. The processing of fibrillar collagen occurs in a stepwise manner. Procollagen genes are transcribed and translated to form procollagen monomeric chains (procollagen precursor). Three of these monomers assemble intoa trimer triple helix. This is aided by disulphide bridge formation. Trimeric procollagen chains are then acted upon by N- and C-terminal proteases (PCP and PNP), to cleave the terminal domains. After this cleavage the collagen units form spontaneously into fibrils. The newly formed fibrils are then covalently stabilized through cross-linking to form a stable mature structure of collagen.

The genes COL1A2, COL3A1, COL6A1, COL6A3, and COL7A1 have been identified as definite TGF-β targets. These are early induced genes in fibroblasts. They were identified by differential hybridization of cDNA array. The transcriptional activation of types I and VII collagen gene expression by TGF-b has been demonstrated. This transcriptional activation of procollagen genes by TGF-β is causing an increased expression of procollagen genes and hence contributing to increased collagen level inOSF.[18,19,20]

2. **ELEVATION OF PROCOLLAGEN PROTEINASES LEVELS**

Procollagen proteinases play an essential role in processing of procollagen precursors

into collagen fibrils, which are soluble. There are two types of proteinases that cleave the N- and C-terminal, respectively – PCPand PNP.

a. Procollagen C-proteinase (PCP)

The PCP and BMP1 have been shown to be the same protein that cleaves the C-terminal of procollagen precursor. TGF-β1 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.

b. Procollagen N-proteinase (PNP)

It cleaves the N-propeptide of procollagen precursor. There are two types of PNPs, PNP I and III, they are classified based on the type of procollagen fibers on which they act. TGF-β-treated cells have been shown to have an elevated level of PNP.[21,22,23,24,25,26,27]

UP-REGULATION OF LOX

The LOX is an essential enzyme for final processing of collagen fibers into a stabilized covalently cross-linked mature fibrillar form that is resistant to proteolysis. The LOX is dependent on copper for its functional activity. Removal of copper leads to a catalytically inactive apoenzyme. The LOX is synthesized as prolysyl oxidase and conversion of this precursor into an active LOX is mediated by BMP1 and takes place in the extra cellular space. 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 tyrosylquinone (LTQ). The LTQ is essential for the reaction mechanism of LOX, i.e. in the formation of cross-links in the collagen fibers.

The expression of LOX is regulated by various factors, among which TGF-β is considered to be an important factor. TGF-β has been found to strongly promote the expression of LOX both at them RNA and protein levels in various cell lines. The LOX activity is important for formation of insoluble collagen due to cross-linking. The process of cross-linking gives tensile strength and mechanical properties to the fibers as well as makes the collagen fibers resistant to proteolysis.[28,29,30,31]

STABILIZATION OF COLLAGEN

Reduce collagen degradation by tannin plays a major role in pathogenesis of oral submucous fibrosis. Large quantities of tannin present in areca nut reduced collagen degradation by inhibiting collagenases and proposed the basis for fibrosis as the combined effect of tannin and arecoline by reducing degradation and increased production of collagen respectively. Collagenase activity was measured with soluble14C-glycine-labeled collagen as a substrate and showed reduced activity in fibroblasts from OSF compared with controls.[32]

Highly fibrogenic cell population in the altered tissue under the influence of local factors such asinterleukin-1 from inflammatory cells also leads to decrease in fibroblast phagocytosis &accumulation of collagen in oral mucosa. (Figure 3)

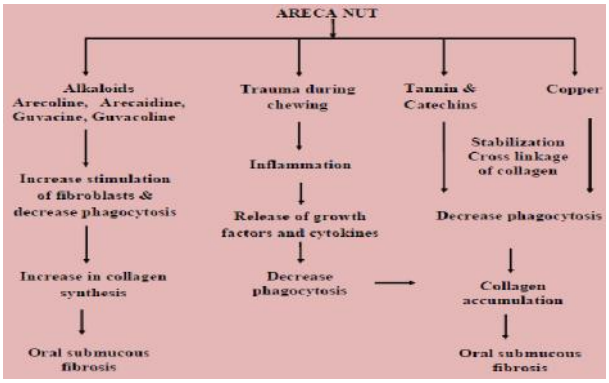

Figure 3: Role of areca nut in pathogenesis of oral submucous fibrosis [13,17,32,53]

ROLE OF COPPER IN OSMF

Arecanut is high in Copper, and its role in pathogenesis of OSF has been subjected to a wide array of studies. The enzyme lysyl oxidase is a copper activated enzyme critical for collagen cross linking and organization of ECM. Salivary Copper is found to increase markedly, following chewing arecanut. This finding indicates that soluble Copper found in arecanut is released into the oral environment and its buccal absorption may contribute to fibrosis of buccal mucosa. This suggests the local effect of Copper in OSF patients.[33,34,35]

The enzyme lysyl oxidase is found to be up regulated in OSF. This is a copper dependent

enzyme and plays a key role in collagen synthesis and its cross linkage. The possible role of copper as a mediator of fibrosis is supported by the demonstration of up regulation of this enzyme in OSF biopsies and in OSF fibroblasts compared to normal fibroblasts grown in culture. The fibroblasts in OSF have not only increased lysyl oxidase activities but also specific growth characteristics. This was evident with the reported cell doubling time of 3.2 days for OSF and 3.6 days for normal fibroblasts.[36,37,38]

CHANGES IN EXTRACELLULAR MATRIX

Increased and continuous deposition of extracellular matrix may take place as a result of disruption of the equilibrium between matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMP). When normal (control) fibroblasts and fibroblasts from OSF patients were subjected to arecoline and arecadine in culture, OSF fibroblasts produced more TIMP-1 protein than normal fibroblasts; mRNA expression of TIMP-1 in OSF firoblasts was also higher.[39]

In the lamina propia and submucosal layertenascin, perlecan, fibronectin and collagen type III were enhanced in early stages of oral submucous fibrosis.In the intermediate stage elastin was extensively and irregularly deposited around muscle fibres, together with the abovementioned molecules. In the advanced stage all those ECM molecules decreased and were entirely replaced by collagen type I only. Their gene expression levels were varied with the progression of fibrosis. Therefore, it is clear that ECM remodeling steps in OSF are similar to each phase of usual granulation tissue formation and maturation.[40]

Malondialdehyde (MDA) is a lipid peroxidation end product with the potential to stimulate fibroblasts and to increase collagen production by 2-3 times. A recent study has reported a significant elevation in serum MDA levels as the grading of OSF progressed. Tissue MDA levels were increased in grade 1 and 2OSF when compared to controls. Yet tissue levels of MDA were found to be reduced in grade 3 OSF when compared to controls, suggesting the utilization of MDA in cross linking of collagen at the advance stage of the disease.[41]

UPREGULATION OF CYCLO-OXYGENASE (COX-2)

Treatment of the buccal mucosal fibroblasts with 80 µg/ml arecoline in culture revealed that COX-2 expression was up-regulated as early as half an hour, indicating this to be an early cellular response to arecoline at transcriptional level. COX- 2 expression started to decrease when the arecoline concentration was increased up to 160 µg/ml, and this may be due to cytotoxicity.[42]

GENETIC POLYMORPHISM

Some genotypes of cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), a negative regulator of T-lymphocyte activation seems to have a susceptibility for various autoimmune diseases. Interestingly, the G allele at position +49 ofexon 1 was found to be significantly associated with OSF compared with controls by Shin Yi et al.[43] Genetic predisposition in pharmacokinetics for toxic substances of betel quid plays a major role in development of the disease. Meanwhile polymorphism of Cytochrome P450 3A gene family is considered as a major determinant of the interindividual variability in chemical pharmacokinetics.

Hence Cytochrome P450had been identified as a genetic biomarker for susceptibility to OSF and authors have further suggested that individuals at genetically high risk for Oral submucous fibrosis could be screened according to the genetic polymorphisms in some exclusive regions of the Cytochrome P450 3A genes. [44]Anotherstudy highlighted that polymorphism in Cytochrome P4501A1 andCYP2E1 may confer an increased risk for OSF. While a different study brought about the fact that CYP1A1 (m1) genotype and (m2)genotype singly acts as a protective factor but in the absence of GSTM1and/or GSTT1 gene significantly alters risk towards the disease.[45,46]

Genes [CYP2B6, CYP2C18, CYP2F1, CYP3A5, microsomalglutathione S-transferase 2 (MGST2), alcohol dehydrogenase (ADH),UDP glucuronosyl

transferase 2B15 (UGT2B15), ADH1C) which were related to the pathway of CYP metabolism were found to be down regulated in all stages of OSF, thereby reducing the ability of CYP to metabolize and clear betel nut substances. This may ultimately contribute to the pathogenesis. [47]

COLLAGEN-RELATED GENES

There are various evidences to 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 procollagengenes by TGF-β suggests that it may contribute to increased collagen levels in OSF.[18]

INFLAMMATORY CYTOKINES

Among the many cytokines implied in the pathogenesis by influencing the synthesis of collagen, Tumor Necrosis Factor-α (TNF-α) is of critical importance. TNF-α mediates multiple functions, out of which the regulation of inflammatory reaction as well as transcription of collagen and collagenase are the most important in the pathogenesis of OSF. Recently it was reported that the homozygous wild genotypeTNF-α2 was significantly associated with an increased risk of OSF and the mutant allele TNF-α2 is about 7 times more efficient in promoter function than the wild allele.

Considering these facts it was suggested a role of TNF-α in OSF pathogenesis through its modulation of collagenmetabolism.[48] According to literature available on growth factors and cytokines, TGF β appears to be the main mediator of the disease and others such as TNF-α, IGF-1, b-FGF and CTGF may contribute to continuous accumulation of collagen with activation of signalling pathways such as ALK5, JNK, SMAD and p38 MAPK.

FIBROGENIC CYTOKINES

Haque MF used a three-stage immunoperoxidase technique to investigate the expression of interleukin alpha (IL-1alpha) and beta, IL-6 interferon (IFN)-alpha, beta and gamma, transforming growth factor beta (TGF-beta), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) in frozen sections of OSF and compared it with that in normal buccal mucosa. The expression of cytokines and growth factors in normal tissues was consistent with their well known distribution and cell of origin, but the intensity and distribution in OSF were all, with the exception of IFN-alpha and gamma, upregulated with strong expression in both the epithelium and underlying connective tissue. Increased expression of fibrogenic cytokines namely TGF-β, platelet derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) were found in OSF tissues compared to normal.[49]

In another study authors investigated the spontaneous and stimulated production of cytokines by peripheral blood mononuclear cells (PBMC) from OSF patients and compared them with genetically-related relatives, Indian and Caucasian control subjects. Results from this study demonstrated increased levels of proinflammatory cytokines and reduced anti-fibrotic IFN-gamma in patients with OSF, which may be central to the pathogenesis of OSF.[50]

EPITHELIAL–MESENCHYMAL TRANSITION (EMT)

Yanjia HU et al conducted a study to identify the genes responsible for its pathogenesis and malignant transformation using oligonucleotide microarray. The expression profiles of 14,500 genes in human oral submucous fibrosis and normal control were analyzed using Affymetrix U133A 2.0 GeneChip arrays. The results revealed that 716 genes were upregulated and 149 genes were down regulated in OSF. Gene Ontology (GO) and relevant bioinformatics tools identified a list of significant differentially expressed genes involved in immune response, inflammatory response and epithelial-mesenchymal transition (EMT) induced by TGF-ß signaling pathway.

Five EMT-related genes including SFRP4, THBS1, MMP2, ZO-1, and CK18 were validated with RT-PCR. Data suggested that

gene abnormalities in immune response, inflammatory response and EMT induced by TGF-ß might play an important role in the pathogenesis and malignant transformation of OSF.[51]Hif-1 α enhances the EMT in vitro and promotes fibrogenesis by increasing expressionof extracellular matrix–modifying factors and lysyl oxidase genes.[52]

HYPOXIA IN ORAL SUBMUCOUS FIBROSIS

With the progression of the disease process of OSMF, the production of collagen type 1 is increased, and the degradation of collagen is reduced by up to 75%. These changes have been mainly attributed to the alteration of collagen-related genes by chemicals present in areca nut, namely arecoline, arecaidine, guvacine, and guvacoline. Extensive fibrosis of the connective tissue causes reduction of vascularity, resulting in subsequent hypoxia in both fibroblasts and surface epithelia. Hypoxia causes atrophy and ulceration of the epithelium by inducing apoptosis.[53]In addition, the overexpression of hypoxia-induced factor-1a is seen in OSMF, which indicates changes in cell proliferation, maturation, and metabolic adaptation, increasing the possibility of malignant transformation.[54]

CHILLIES:

The use of chillies (Capsicum annum and Capsicum frutescence) 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.[55]

ROLE OF INFECTION

Jalouli J et al investigated the prevalence of HPV, herpes simplex virus (HSV), and Epstein–Barr virus (EBV) DNA in two groups of patients using betel quid with tobacco, those with OSMF(n=12) and those with OSCC(n=62).DNA was extracted from all the samples and viral genome was examined by PCR/DNA sequencing. HPV-positive samples were analyzed separately for the high-risk types HPV 16 and 18.The result from this study showed HPV DNA, HSV DNA, and EBV DNA were detected in 11 (91%), 1 (8%), and 3 (25%) of the 12 samples from patients with OSMF compared with 15 (24%), 3 (5%), and 18 (29%), respectively, from 62 patients with OSCC. HPV 16 and 18 DNA was detected in 8/12 (67%) in the OSMF group and 10/62 (16%) in the OSCC group.HPV DNA, HSV DNA and EBV DNA were detected from patients with OSMF.[56]

Rajendran R evaluated the role of H pylori in the etiology of mucosal inflammation, a condition that compounds the morbid state associated with oral submucous fibrosis (OSF).Role of H pylori in the etiology of mucosal inflammation, a condition that compounds the morbid state associated with OSMF was assessed using Rapid urease test (RUT) of plaque samples to estimate the H pylori bacterial load. A positive correlation exists between RUT reactivity and the frequency of mucosal inflammation.[57]

Ariyawardana A (2007)[58] determined the prevalence of oral yeast carriage in patients with OSMF and to compare the carriage with the normal individuals. The prevalence of oral yeast carriage in patients with OSMF as compared to the carriage with the normal individuals was assessed. The carriage of yeast in the OSMF group was not statistically significant compared with the control group. C. dubliniensis was isolated from the oral cavities of both OSMF patients and healthy individuals.

ROLE OF AUTOIMMUNITY IN OSMF

Canniff et al (1981) that the human leukocyte antigens (HLA) A10, B7 and DR3 occurred significantly more frequently in OSF, is important. It is found that anti neuclear antibody (ANA) 23.9%, smooth muscle antibody (SMA) 23.9% and gastric-parietal cell antibody (GPCA) 14.7% positive in OSF patients compare with healthy control subjects. A recent study has revealed higher haplotype frequencies in pairs HLA B51/Cw7 and B62/Cw7 in OSF patients. Two new HLADRB1 alleles were identified by sequencing-based typing and named as HLADRB1-0903 and DRB1-1145.[6,59]

CONCLUSION: Oral submucous fibrosis is a well recognized disease for its malignant potential. OSMF is one of the poorly

understood and unsatisfactorily treated oral diseases because of its multifactorial etiology. Constituents of areca nut are well established cause of stimulation and proliferation of fibroblasts. Evidences suggest that OSMF is multi-factorial, with certain effects on specific subpopulations of fibroblasts, genetic predisposition and molecular mechanisms, which could render the oral mucosa more susceptible to chronic inflammatory changes on exposure to carcinogens. After having a glance of vast literature on etiology of OSMF it can be said that there is hope for further detail evaluation of etiopathogenesis as well as management of this premalignant condition.

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