(e) ISSN Online: 2321-9599
(p) ISSN Print: 2348-6805

REVIEW ARTICLE

PATHOGENESIS IN THE MALIGNANT TRANSFORMATION OF ORAL SUBMUCOUS FIBROSIS: A REVIEW

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ABSTRACT:
Oral submucous fibrosis (OSMF) is a disease of the oral mucous membrane predominant in South Asia. With the increased availability and popularity of areca nut, the incidence of the disease has increased. The manufacturers have managed to make the tiny sachets more appealing and attractive for all age groups. OSMF draws attention because the disease has no known cure and the number of areca nut chewers is increasing without any check, despite of the bans setup by the governments curbing the sale of such products. There are millions of people suffering from the disease. Besides arecanut, autoimmunity is also a causal factor. OSMF has a known malignant potential, with its transformation to oral squamous cell carcinoma (OSCC) documented. The article reviews the mechanism behind malignant transformation of OSMF and henceforth the newer treatment options that are being studied.

Key words: Oral submucos fibrosis, squamous cell carcinoma

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This article may be cited as: Kaur K, Kaur J, Singh S, Kaur N, Kaur K. Pathogenesis in the malignant transformation of oral submucous fibrosis: a review. J Adv Med Dent Scie Res 2016;4(4):27-31.

Access this article online	
Quick Response Code	Website: www.jamdsr.com
	DOI: 10.21276/jamdsr.2016.4.4.7

INTRODUCTION:

Oral mucous membrane is a unique tissue which is continuously exposed to various kinds of stresses such as heat, cold, micro organisms, chemicals and mechanical irritation in the process of food intake. In response to these stresses, both epithelial and connective tissue layers of the oral mucosa exhibit acute and chronic reactive changes.[1] Oral submucous fibrosis (OSMF) is a well recognized potentially malignant disorder of the oral mucosa predominantly seen in people of South and South-East Asia. It is a chronic progressive disorder that involves the oral mucosa and occasionally the pharynx and upper third of the oesophagus[2] and is characterized by marked stiffening of oral mucosa and development of fibrous bands, loss of elasticity of the mucosa resulting in a progressive restriction of mouth opening. Besides being regarded as a precancerous condition, it is a serious debilitating disease with cases progressing into oral squamous cell carcinoma (OSCC). Paymaster first described the malignant potential of OSMF in 1956, and the rate has been estimated to be 7-13% recently. With its high probability of malignant transformation, OSMF is considered a disorder with a significant mortality rate.[2]

It is believed that oral cancers arising in OSMF constitute a clinico-pathologically distinct disease and those differences are attributed to the differential mechanisms of arecanut carcinogenesis. While one study reported that most of such patients are younger males with better prognostic factors, another retrospective study in China has come up with contradictory findings that OSCC originated from OSMF is clinically more invasive with a relatively worse prognosis than OSCC not originated from OSMF.[2,3] Simultaneous occurrence of oral leukoplakia (OLE) and OSMF carry a higher risk for OSCC and experience early malignant

transformation than those with either OLE or OSMF alone.[4]

The main aetiological agent causing OSMF is confirmed as arecoline in arecanut. Areca nut chewing is known to cause local trauma and injury to the oral mucosa due to its abrasive nature. This could be more severe in users of pan masala and gutkha due to their fine particulate nature, with the high probability of particle adhesion to the traumatized mucosa, leading to morphological changes and membrane damage. Areca nut, present in these mixtures, can disturb collagen homeostasis, cause crosslinks and accelerate the onset of OSMF, a collagen-related disorder, in habitual chewers. This continuous local irritation by panmasala, gutkha or areca nut can lead to injury-related chronic inflammation, oxidative stress and cytokine production. Oxidative stress and subsequent reactive oxygen species (ROS) generation can induce cell proliferation, cell senescence or apoptosis, depending upon the level of ROS production. During chronic exposure, these events can lead to preneoplastic lesions in the oral cavity and subsequently to malignancy.[5]

Affected users experience a burning sensation of the oral mucosa, occasional mucosal ulceration, a peculiar marble-like blanching of the mucosa and palpable fibrous bands of the buccal mucosa, soft palate and lips.[3] The major alkaloid of arecanut, arecoline is well known for its different cytotoxic and genotoxic properties. And arecanut alone, even when not combined with tobacco has a definite potential for carcinogenicity.[6] The ultimate carcinogen in areca related oral carcinogenesis, according to a recent study is Arecoline N–oxide, the active metabolite of arecoline.[7]

Betel quid (BQ) consists of a mixture of areca nut (Areca catechu), slaked lime (calcium oxide and calcium hydroxide), catechu (Acacia catechu) and several condiments according to taste, wrapped in a betel leaf (Piper betle). Almost all habitual chewers use tobacco with or without the betel quid. In India, most habitual chewers of BQ add tobacco while in some countries, such as Papua New Guinea and China, tobacco is not added.[3] In the last few decades, small, attractive and inexpensive sachets of betel quid substitutes have become widely available. Aggressively advertised and marketed, often claimed to be safer products, they are consumed by the very young and old alike, particularly in India, but also among migrant populations from these areas worldwide. The product is basically a flavoured and sweetened dry mixture of areca nut, catechu and slaked lime with tobacco (gutkha) or without tobacco (panmasala). It has been estimated that, worldwide, ~600,000,000 people chew areca nut. BQ chewing was already a socially well accepted practice and the introduction of tobacco reinforced this practice. Condiments, sweetening agents and spices may be added according to individual preferences.[5]

BQ chewing has been related mainly to oral, pharyngeal and oesophageal cancer. Chewing of tobacco with BQ results in high exposure to carcinogenic tobacco-species nitrosamines (TSNAs), to ~1000 mg/day, compared with ~20 mg/day in smokers, as well as leading to exposure to nitrosamines derived from areca nut alkaloids. The carcinogenic TSNAs N¢-nitrosonornicotine (NNN), 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosoanabasine (NAB), as well as the volatile nitrosamines N-nitrosodimethylamine and N-nitrosodiethylamine, have been detected in the saliva of chewers of BQ with tobacco.[3]

TSNAs undergo metabolic activation by cytochrome P450s and other enzymes. NNK, a major carcinogenic TSNA, is activated by either methylene hydroxylation to generate an intermediate that decomposes to a DNA-methylating agent, resulting in the formation of 7-methylguanine, O6-methylguanine (O6-MeG) and O4-methylthymidine in DNA or via methyl hydroxylation to form bulky pyridyloxobutyl DNA adducts. NNK is also converted metabolically to 4-(methylnitrosamino) - 1-(3-pyridyl)-1-butanol, which can also be activated by hydroxylation to yield methyl and pyridylhydroxybutyl adducts in DNA. 2¢-Hydroxylation of NNN, another important TSNA, can give rise to the same intermediate as is formed by methyl hydroxylation of NNK, resulting in pyridyloxobutylation of DNA The areca nut-specific nitrosamines (ASNAs) N-nitrosoguvacoline (NG) and the carcinogenic 3-(methyl-N-nitrosamino) propionitrile (MNPN) were also detected in the saliva of chewers of BQ without tobacco. ASNAs were not detected in BQ containing areca nut. Nitrosation of BQ with nitrate and thiocyanate in vitro at neutral pH resulted in the formation of NG. Nitrosation of arecoline at neutral pH yielded approximately four times more NG than at acidic or alkaline pH. Hence the reported presence of ASNAs in the saliva of BQ chewers could arise from their formation during chewing of BQ.[5]

Also arecoline reduces p21 and p27 levels and thereby facilitates G1/S transition of the cell cycle and lead to error-prone DNA replication[8] p21 and p27 are known to induce cell cycle arrest. With the use of Western blot analysis a different study

reports that arecoline regulates S and/or G2/M cell cycle related proteins as p21, cdc2 and Cyclin B1and they in turn play a vital role in different stages of arecoline mediated carcinogenesis.[9] As a response to arecoline, Brca1 and Brca2 tumour suppressor gene expression is reduced[10] and human telomerase reverse transcriptase (hTERT) is over expressed in OSMF [11] and both events may contribute to malignant transformation of OSMF.

Arecoline upregulates αvβ6 expression and thereby promotes keratinocyte migration and induces invasion.[12] COX-2 gene in fibroblasts is upregulated as a response to arecoline at the early stage of OSMF and it is more pronounced in OSCC when compared to premalignant lesions.[13] The arecanut carcinogenicity in part can be attributed to exposure of oral mucosal cells to ROS, methylating agents and reactive metabolic intermediates from arecanut and its constituents. ROS produced during auto oxidation of arecanut polyphenols in betel quid chewer's saliva, play an important role in the initiation and promotion of oral cancer, meanwhile arecanut specific nitrosamines produced by nitrosation of areca alkaloids are found to induce tumors in experimental animals. These cytotoxic agents have the potential to damage cellular DNA and cause subsequent carcinogenesis.[2]

Hypoxia plays a significant role in malignant transformation and progression of OSF. A study based on this hypothesis has identified that hypoxia-inducible factor 1 α (HIF-1α), a known transcription factor induced by hypoxia, is upregulated at both protein and mRNA levels. HIF-1 α is a key regulator of the cellular response to hypoxia and is associated with activation of many hypoxia-inducible genes such as genes encoding for VEGF. VEGFs are a family of potent pro-angiogenic factors that stimulate endothelial cell proliferation and the resulting neo angiogenesis is a crucial event for the development, progression, and metastasis of malignant tumours.[2]

The proliferating activity as denoted by proliferating cell nuclear antigen (PCNA) index was higher in OSMF epithelium than normal oral mucosa. And also a significant increase in the same is noted in the dysplastic OSMF when compared with the non-dysplastic group. Compiling all these findings, the study suggested that the increased PCNA index may correlate with the increased malignant transformation potential. The important molecules in G2/M phase namely Cyclin B1, p34 (cdc2) and p-survivin, play a key role in carcinogenesis by influencing mitosis. It is reported that their expression is higher in OSF than in normal and a significant difference was present in expression between OSMF and OSCC.[13,14] p63 in concert with p53 regulates cell proliferation and differentiation. The significant increase in expression of p53 and p63 proteins in OSCC and OSMF suggests their role as surrogate markers of malignant transformation.[2,15] The FHIT gene, a member of the histidine triad gene family, is a tumor suppressor gene exhibiting deletions in majority of human cancers. FHIT expression is found to be decreased in OSMF and more significantly in carcinoma arising in OSMF, while MDM2 expression is increased in OSMF and more in carcinoma arising in OSMF. Both the loss of FHIT and overexpression of MDM2 are supposed to play an important role in carcinogenesis of the disease.[16] As over expression of Loricin and loss of Cytochrome P450 3A5 (CYP 3A5) are involved in the change of defending ability of epithelium, it is suggested that they may be responsible for carcinogenesis of OSMF. Chronic OSMF which is significantly associated with hypermethylation of E-cadherin and COX-2 which is a mutation of epigenetic level, hence more prone to malignant transformation. However, COX-2- 765 G>C polymorphism was identified as a protective factor against OSCC development. TNF α (-308) genetic polymorphism shows no association with OSCC arising in OSMF patients.[2,17]

Endogenous angiogenic promoters as iNOS, b-FGF, TGF-β, PDGF, and HIF-1α are found to be expressed in OSMF and play an important role in maintaining the vascularity of underlying connective tissue. This angiogenesis in turn plays an important role in tumour proliferation, once the malignant transformation takes place.

A study based on inducible nitric oxide synthase (iNOS) reports a significant vasodilatation in the OSF cases and also the enhanced expression of iNOS which is a powerful cytotoxic and genotoxic agent is assumed to have an effect on epithelial keratinocytes leading to epithelial thinning in OSMF.[18]

Arecoline toxicity may cause reduced vascularity in OSMF.[19] Vascularity of the mucosa is known to reduce with advanced fibrosis and starts to increase in the juxtaepithelial area once dysplasia appears in the epithelium. That may be part of angiogenesis related to carcinogenesis. These facts were further clarified with a recent study of morphological analysis of mucosal vasculature in OSF. The study identified that the vascularity was increased in early OSMF and reduced in advanced OSMF, with the suggestion that inflammation may play a role in the early stages while progressive fibrosis may

predispose to atrophy of the epithelium and subsequent malignant changes. It has been observed that the vascularity increased progressively from normal to premalignancy and malignancy, there by emphasizing the importance of angiogenesis in tumor development and progression.[2]

αvβ6 integrin is known for its ability to promote tissue fibrosis as well as carcinoma invasion and its expression is high in OSMF. The effect of epithelial mesenchymal transition, in OSMF, keratinocytes in the epithelium could be altered in such a manner that the altered keratin profiles would influence the disease pathogenesis, as well as the predisposition to malignant transformation.[20]

There is evidence to suggest that trace metals such as copper may play an important role in the development and progression of neoplasia.[21] The mutagenicity of trace metals such as copper has been well documented in head and neck cancer as well in cancers of the gastrointestinal tract, pancreas and cervix. The exact mechanism of copper-induced mutagenesis is not fully understood. Copper-induced DNA damage has been reported and there is evidence to suggest that copper may bind to the protein product of p53, resulting in alteration of its conformation.[13] p53 aberrations have been reported in OSMF tissues.[22] p53 stabilization, which may be critical in the progression of potentially malignant lesions to squamous cell carcinoma, may thus arise from the DNA damage inflicted by chewing copper-containing areca nut. Patients with OSCC are reported to have a higher salivary copper level than patients with OSMF. A recent study has revealed a marked, progressive and significant increase in serum copper levels in OSMF and OSCC groups as compared to the normal group. The high copper content in arecanut influences tumour angiogenesis by activating several angiogenic factors such as VEGF, TNF α, IL-1 and β- FGF. These molecules stimulate endothelial cell proliferation and activation.[21] Lysyl oxidase (LOX) is an essential copper dependant enzyme; for final processing of collagen fibers into a stabilized covalently crosslinked mature fibrillar form that is resistant to proteolysis. Chewing areca nut increases copper levels which in turn stimulates fibrogenesis through up-regulation of LOX activity. Zinc may play a role in chelating copper and correct the imbalance of excess copper proposed as a causative factor.[23]

There is clinical and experimental evidence on OSMF to support an autoimmune etiology e.g., the high incidence of anti-nuclear antibodies together with auto-antibodies to gastric parietal cells, thyroid microsomes, reticulin and smooth muscle noticed in this disease. The increased frequency of HLA haplotypic pairs A10/DR3, B8/DR3 and A10/B8 in OSMF and scleroderma suggests an MHC mediated immunological derangement operating this disease. There is increase in evidence that immunoregulatory aberrations are primarily involved in the pathogenesis of autoimmune diseases. In man, evidence is accumulating that immune response genes are associated with the HLA complex, indicating a relation between autoimmunity and HLA type.[24]

Literature supports the use of several medical interventions,

including micronutrients, anti-oxidants, proteolytic enzymes, immune modulators (mainly steroids), and agents that promote blood flow as being useful in the management of OSMF.[25] Vitamins A, B complex, C and E have been tried alone or in combination with other agents.[26] One of the pathogenic mechanisms of areca nut has been attributed to the generation of reactive oxygen species, free radicals and peroxidases. Naturally occurring or synthetic anti-oxidants include beta carotene, lycopene, tea pigments, aloe vera, curcumin and spirulina. Marked improvement in mouth opening has been seen with oral lycopene with intralesional corticosteroids.[27]

Human placenta, interferon gamma, COX 2 inhibitors, hyaluronidase, various vasodilators such as pentoxifulline, nylidrin hydrochloride, buflomedial hydrochloride and danxual koukang, and isoxsuprine have been used in the treatment of OSF according to various studies.[28]

CONCLUSION

Malignant transformation of OSMF remains to be a concern. Future studies need to be conducted for more information about the malignant transformation, to achieve more success in the management arena. The easy availability of areca nut products also poses a hindrance; so, patients should be educated to reduce or stop the use of such products.

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Source of support: Nil **Conflict of interest:** None declared