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Oral sub-mucous fibrosis: realities of etiology

Fibrose de submucosa oral: a realidade da etiologia

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Abstract

Oral sub-mucous fibrosis (OSMF) is a well known potentially malignant condition resulting commonly due to the use of areca nut. Areca nut chewing is a primordial tradition in Asian countries. It is also a psychoactive essence. With the emergence of commercial pan masala and gutkha, witness of massive growth in the sales of these products, with huge worldwide export market developed. Various components in this quid have its own mechanism of action resulting in OSMF. Other causes proposed were chillies, misi, nutritional deficiency, genetic predisposition, immunologic aspects, infection and saliva. This review attempts to give an overview about the postulated etiologies and its role in causation of OSMF.

Keywords: OSMF. Areca nut. Gutka. Multifactorial.

Resumo

Fibrose de submucosa oral (FSMO) é uma condição potencialmente maligna muito conhecida resultante geralmente do uso da noz de areca ou betel. Mastigar a noz de betel é uma tradição primordial nos países asiáticos. É também uma essência psicoativa. O surgimento comercial da pan masala e da gutkha, aliado ao enorme

crescimento nas vendas desses produtos, possibilitou um grande mercado de exportação em todo o mundo desenvolvido. Vários componentes neste material tem seu próprio mecanismo de ação, resultando em FSMO. Outras causas propostas foram pimentões, misi, deficiência nutricional, predisposição genética, aspectos imunológicos, infecção e saliva. Esta revisão procura dar uma visão geral sobre as etiologias postuladas e seu papel na causa da FSMO.

***Palavras-chave**: FSMO. Noz de areca. Gutka. Multifatorial*

Introduction

The available scientific literature at present makes it apparent that regular use of areca nut (1) and gutka (2) is the major etiological factor in the causation of OSMF. Areca nut, often referred to as betel nut and commonly called as supari, is the fruit of areca catechu palm tree which is native of South Asia and Pacific islands. The fruit is green in color and the seed or endosperm is consumed fresh, boiled, or after sun drying or curing. In India, areca nut is chewed by itself or in the form of commercial preparations like supari, mawa, paan masala, and betel quid with or without tobacco; the frequency of which can vary from 15-20 times/day for varying duration (1). OSMF shows an age predilections from 10-70 years with the mean age between 20-40 years (3). Reports of gender ratio vary, but seem to favor a female predominance (4, 5). Whereas some studies denote male predominance (6, 7).

Clinical importance of the disease is mainly because it is a disease that results from the use of betel-nut chewing in Indians as well as other nationalities adopting the oral habit of betel-nut chewing, and it is generally accepted to be a premalignant condition of the oral cavity (8).

The etiology is multifactorial and still uncertain as no conclusive etiological factor has been identified though plenty of data has been generated on various aspects of the disease. Following factors have been postulated as etiologies for OSMF: areca nut chewing, ingestion of chilies, misi, nutritional deficiency, immunological, genetic, infectious agents and saliva.

Areca nut (betel nut) chewing (flow chart)

The use of areca nut is thought to be the most important etiological agent. Areca nut is the endosperm of the fruit of the areca catechu palm (9). With the emergence of commercial pan *masala* and *gutkha* about three decades ago, witness of massive growth in the sales of smokeless tobacco and areca nut products, with huge worldwide export market developed (10). The leaf of piper betel contains betel oil containing two phenols (55%), betel phenol and eugenol. They also contain tannins (flavanols), sugar, vitamin C, starch and diastase. Eugenol is a Central nervous system (CNS) stimulant, a sialogogue and has a local anesthetic effect on oral mucosa and it is cytotoxic to oral mucosal fibroblasts in vitro at concentrations exceeding 3 mmol/L whereas it has the potentially protective effect of inhibiting xanthine oxidase activity and lipid peroxidase at levels below this (11, 12, 13).

Effect of arecoline histologically on the palatal and buccal mucosa of twenty eight Wistar rats applied for varying periods of time found that the OSMF may be produced by the habit of chewing betel nut (14). Extracts of betel nut exacerbate the OSMF initiated by chronic inflammation by stimulating matrix synthesis and the proliferation of submucosal fibroblasts and also by stabilizing the collagen that they produce (15). Smoking and alcohol consumption habits have no effect in the development of OSMF, but their addition to areca nut chewing can be a risk for OSMF (16).

Commercially freeze-dried products such as pan masala, guthka, and mawa had higher concentrations of areca nut per chew and appeared to cause OSMF more rapidly than the self prepared conventional betel quid, which contains smaller amounts of areca nut (17). Preparations from varieties of areca catechu nuts at a concentration of 10 μg/ml stimulated collagen synthesis by approximately 150% in micro well cultures of human fibroblasts, using a pulse of 3H-proline and subsequent analysis of the cultures for radioactive collagen (18).

Arecoline elevated the mRNA and protein expression of cystatin C and they are consistently up-regulated in a variety of fibrotic diseases, in a dose-dependent manner in persons with OSMF

(19). Keratinocyte growth factor-1, insulin like growth factor-1, and interleukin 6 expression, were significantly up regulated in persons with OSMF due to areca quid chewing and arecoline may be responsible for their enhanced expression (20, 21, 22). Western blot testing performed on OSMF specimens showed to have a higher TIMP-1 expression. It was found that arecoline acted not only as an inhibitor on gelatinolytic activity of MMP-2, but also a stimulator for TIMP-1 activity. These synergistic effects may contribute to the extra cellular matrix molecules components accumulation in the areca quid associated OSMF (23).

Areca nuts had been shown to have a high copper content, and chewing areca nuts for 5-30 minutes significantly increased soluble copper levels in oral fluids. Copper acts as an initiating factor in persons with OSMF by stimulating fibrogenesis through up-regulation of copper dependent lysyl oxidase activity (24). Copper content in various betel quid ingredients has been reported to range from 3 to 108 mg/g in areca nut and from 8 to 53 mg/g in pan masala (25, 26, 27). The iron levels measured were 75 ng/g in areca nut, 132 ng/g in betel leaf, 5.2 ng/g in catechu and 22 ± 256 ng/g in slaked lime samples (28, 29).

Significant superoxide anion production, assayed by cytochrome C reduction and lipid peroxidation by formation of thiobarbituric acid-reactive substances, was demonstrated in normal human oral keratinocytes following exposure to commercially available gutkha and pan masala extracts (30). Thus, some of the cytotoxic effects of these chewing products appear to be mediated through production of Reactive Oxygen Species (10).

Even after quitting the betel nut chewing for about 13 years, the clinical and histological features of oral mucosa showed signs and symptoms of OSMF. It was then concluded that once OSMF was induced by the habit of chewing betel nut, the reversal of the disease after cessation of the habit could not occur (31).

Ingestion of chilies

Chilies can damage the cells of the mucosa and if this is continuous, it probably causes chronic inflammation, which leads to the formation of excessive fibrosis (32). Experimental evidence demonstrated that by applying capsaicin, the active irritant in chilies to rat palates rendered limited connective tissue response but in protein depleted or vitamin B-deficient animals, the connective tissue response with fibroblast proliferation was more widespread and extensive (33).

A study in Mexico and South America evidenced that OSMF was not seen in the population, where the dietary intake of chilies equals or even exceeds that in India (34).

Misi

In a study of 30 OSMF cases in Eastern Uttar Pradesh, a substance called "MISI" was constantly being used by female villagers as a cosmetic to keep their teeth clean and shiny. Misi was a black colored powder containing various chemical substances like washing soda, borax, and powdered alum, charcoal of myrobalan and fullers earth in varying proportions. In their study group, 21 out of 24 females used misi. So it was considered to be one of the factors causing OSMF in that region (35).

Nutritional deficiencies

A subclinical vitamin B complex deficiency and vitamin A deficiency had been suspected as etiological factor of OSMF. The deficiency could be precipitated by the effect of defective nutrition due to impaired food intake in advanced cases (36).

Cytochrome oxidase is an iron dependent enzyme which is required for the normal maturation of the epithelium. In iron deficiency state, the levels of cytochrome oxidase are low, consequently leading to epithelial atrophy. An atrophic epithelium makes the oral mucosa vulnerable to the soluble irritants. Body iron absorption is controlled by the duodenal mucosa which allows the intake of appropriate quantities of iron to balance exactly the required small daily iron loss. If these iron losses are amplified by the disease or if dietary intake and absorption are impaired, a negative iron balance will result. The effects of this negative balance are counterbalanced for a short duration time by mobilization of body iron stores, resulting in depletion of tissue iron and the serum iron falls resulting in failure of iron supply to the bone marrow. Lack of iron in tissues causes improper vascular channel formation resulting in decreased vascularity. This leads to derangement in

the inflammatory reparative response of the lamina propria resulting in defective healing and scar formation. Thus, the cumulative effect of these initiating and promoting factors leads to further fibrosis, which is a characteristic of OSMF (37).

A significant depression in hemoglobin, serum iron, serum protein and total iron binding capacity showed significant changes in the OSMF patients (38). OSMF appeared to be an altered oral mucosal response following prolonged period of chronic deficiency of iron and/or vitamin B complex especially folic acid. Hypersensitivity caused by local irritants and the resultant persistent juxta-epithelial inflammatory response act as the initiating factor leading to a defective inflammatory reparative response, culminating in fibrotic healing (39). OSMF was reported to be higher in patients with deficiency of vitamin A, B, C and multiple vitamins (40).

Alterations in the expression of retinoic acid receptor beta (RARbeta) were observed suggesting their association with high risk of transition to malignancy (36). Although most interests have been shown in the role of iron in the causation of OSMF, other nutritional factors also may be involved in the pathogenesis of OSMF. Deficiencies in folic acid, pyridoxine, and vitamin may be secondary to that of iron, and hence difficult to estimate. Studies from India and Pakistan showed that patients with oral and oropharyngeal cancers have subnormal levels of serum vitamin A and beta-carotene. Intervention trials with beta-carotene and vitamin A in patients with oral precancers have resulted in substantial regression of the lesion (41-47).

Immunologic

Several characteristics indicate that OSMF may be an autoimmune disease. The most remarkable characteristics that strongly emphasis OSMF to be of autoimmune origin or female bias, the age of onset, incidence of autoantibodies, alteration in humoral immunity and the involvement of DR locus in the genetic predisposition. Besides these features, OSMF resembles scleroderma histologically; the similarities include epithelial atrophy, dermal fibrosis associated with a chronic inflammatory cell infiltrate, an increased frequency of HLA DR3 and the haplotypic pair B8/D3 (48-52).

Raised frequencies of A10 and DR3 supporting the concept that OSMF is a chronic autoimmune disease, initiated by constituents of betel nut (50). Elevated levels of serum globulin and immunoglobulin IgG, states the possibility of OSMF being an autoimmune disorder (53). Estimation of major immunoglobulin profile in OSMF by radical immunodiffusion proved that the severity of OSMF was directly proportional to the estimated elevated levels of major immunoglobulins (54). The number of high affinity rosette forming cells (HAFC) was found to be significantly decreased and levels of serum IgA, IgD and IgE were found to be elevated both in OSMF indicating that OSMF can be an intermediary state in the malignant transformation of normal cells (52). Serum IgA, IgG and IgM levels were elevated significantly elevated in OSMF and circulating auto-antibodies and tissue deposited antibodies were also found in 33% and 44% of cases respectively. Further studies are also required to ascertain the role of cellular immunomechanism and genetic parameters in OSMF (55).

Increased evidence of CD4 and HLA-DR positive cells and high ratio of CD4 to CD8 in OSMF suggests an ongoing cellular immune response (56). Patients with OSMF had a higher frequency of the G allele at position +49 on exon 1 of cytotoxic T lymphocyte associated antigen 4 (CTLA-4). CTLA-4 polymorphism had also been associated with certain autoimmune diseases such as SLE, IDDM, Graves' disease, Hashimoto thyroiditis, multiple sclerosis and rheumatoid arthritis (57).

The presence of immunocompetent cells and the high ratio of CD4 to CD8 results in an either of direct stimulation from exogenous antigens, such as areca alkaloids, or of changes in tissue antigenicity that leads to an autoimmune response (58). Frequency of autoantibodies like antinuclear (ANA), antismooth muscle (SMA), antigastric parietal cell (GPCA), antithyroid microsomal (TMA), and antireticulin antibodies by an indirect immunofluorescence technique (for ANA, SMA, and GPCA) were higher in OSMF patients (59).

Spontaneous and stimulated production of cytokines like interleukin-1beta (IL-1beta), interleukin-6 (IL-6), interleukin-8 (IL-8), tumour necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) was studied by peripheral blood mononuclear cells (PBMC) from OSMF patients. The results showed that a significant differences in the stimulated versus non-stimulated levels of IL-1beta, IL-6, IL-8 and TNF-alpha but not of

IFN-gamma production. So, increased levels of pro-inflammatory cytokines and reduced antifibrotic interferon gamma (INF-γ) in patients with OSMF may be central to the pathogenesis of OSMF (60).

Genetic

Although hypersensitivity to chili and betel quid is often explained as a common factor in the development of OSMF, it was difficult to understand why the disease was not seen in Mexico and South America, where the diet including chili intake equals or even exceeds that in India or the Far East. A proper genetic point of view is vital to explain the condition (61).

Major histocompatibility complex class I chain-related gene A (MICA) has a triplet repeat (GCT) polymorphism in the transmembrane domain, resulting in 5 distinct allelic patterns. In particular, the phenotype frequency of allele A6 of MICA in subjects with OSMF was significantly higher and suggested a risk for OSMF (62). Investigators found a high frequency of mutations in the APC gene and low expression of the wild-type TP53 tumor-suppressor gene product in patients with OSMF, providing some explanation for the increased risk of oral squamous cell carcinoma development in patients with OSMF (63). Insertion/deletion 5A polymorphism in the promoter region of the matrix metalloproteinase-3 gene, results in different transcriptional activities, had also been found in persons with OSMF but not in those with oral SCC (64). Insertion/deletion 2G polymorphism in the promoter of the matrix metalloproteinase-1 gene has been implicated in oral squamous cell carcinoma but not OSMF (65).

Infectious agents

The prevalence of human papilloma virus (HPV), herpes simplex virus (HSV), and Epstein–Barr virus (EBV) DNA in OSMF and oral squamous cell carcinoma (OSCC) groups of patients using betel quid with tobacco was studied. 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 results showed that 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. The difference between presence of HPV DNA in OSMF and OSCC groups was statistically significant, while the difference between HSV and EBV DNA content in OSMF and OSCC groups was insignificant (66).

Saliva

OSMF was considered as a local coagulopathy, produced by thrombin like fibrin producing factor in saliva. Fibrin producing factor acting locally, produced fibrin glue phenomenon in the sub-mucous zone of oral cavity and acting systematically, produced cryofibrinogen, which gets attached to submucous zone through the action of fibronectin (67).

Conclusions

Multifactorial etiologies have been proposed for OSMF but the most accepted is to be the areca nut. But still the investigations and researches are in rummage around to rule out the molecular basis for root of this disease. In the future era, many unanticipated facts may be exploding which could further substantiate fatal nature of this condition. Proper treatment planning that is in terms of proper history, counseling of the patient to quit the habit and selection of the drug can decrease the severity but cannot completely resolve this condition. So prevention of OSMF is better than cure.

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