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Copper in oral submucous fibrosis: implications in clinical trial

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Abstract

Oral sub mucous fibrosis (OSMF) is a chronic, debilitating, potentially malignant disorder caused primarily due to areca nut chewing. Burning sensation of oral cavity and reduced mouth opening are the hallmark of this disease. The etiopathogenesis of this disorder remains unclear. Several trace elements have been implicated in the pathogenesis of OSMF. In this review, the role of copper in OSMF has been summarized emphasizing various molecular pathways affected by this trace element. Available literature regarding serum, salivary, cytological and tissue levels of copper in OSMF has been updated. Including serum copper levels as an outcome in clinical trials assessing medicinal interventions may prove an attractive concept.

Keywords: Copper, Serum copper, Oral submucous fibrosis, OSMF, OSF

Introduction

Oral sub mucous fibrosis (OSMF) is defined as "insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx, occasionally preceded by vesicle formation, always associated with juxtaepithelial inflammatory reaction followed by a fibroelastic change of lamina propria with epithelial atrophy leading to stiffness of the oral mucosa, trismus and inability to eat".[1] It is a potentially malignant disorder caused primarily due to areca nut chewing which is commonly consumed in form of smokeless tobacco products. OSMF is characterized by restricted mouth opening, burning sensation on having hot and spicy food and stiffness and blanching of the oral mucosa. The presence of fibrous bands in lips, cheeks and soft palate is a hallmark of the disease. The disease may extend over time to include the oropharynx and the upper third of the esophagus. Recent epidemiological data indicates a rapid rise of OSMF cases in India from an estimated 250,000 cases in 1980 to 14 million cases in 2010.[2] The prevalence of OSMF is found to be 6.42 per 1000, and the male-to-female ratio, 4.9:1.[3] The malignant transformation rate of OSMF has been reported as 2.3-7.6% in the Indian context[3,4] and up to 27% over many years.[5]

A number of etiological factors have been implicated in the pathogenesis of OSMF such as areca nut chewing, ingestion of chilies, genetic and immunologic processes, nutritional deficiencies, and other factors.[6] Now, conclusive evidence exists through a number of epidemiological surveys, case-series reports, large sized cross sectional surveys, case-control studies, cohort and intervention studies that areca nut is the main etiological factor for OSMF.[7-10] Most accepted pathogenesis of this chronic, insidious disease is that it is primarily 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. It is characterized by a juxta-epithelial inflammatory reaction followed by a fibroelastic change in lamina propria and associated epithelial atrophy.[11]

Copper in the pathogenesis of OSMF

Copper is an essential trace metal for the function of several key enzymes involved in human metabolism. Areca products contain a high level of copper (mean 302 nmol/g) when compared to other commonly eaten nuts (22–173 nmol/g) and that soluble copper is released into whole mouth saliva following chewing areca for 5–30 min.[12] Local factors such as site of quid placement, composition of the quid, length of time chewed, consistency of quid, and whether saliva is expelled or swallowed after chewing, can affect the uptake of copper into the epithelium

Copper has been implicated in the pathogenesis of several fibrotic conditions. The role of copper in the pathogenesis of OSMF is considered due to discovery of a high copper content in areca nut.[13] Copper dependant enzyme lysyl oxidase (LOX) is critical for collagen cross linking and organization of extracellular matrix.[14] In OSMF, there is increased cross-linking of the collagen, resulting in increased insoluble form. This is facilitated by increased activity and production of a key enzyme LOX. Lysyl oxidase has been implicated in other fibrotic disorders such as hepatic and pulmonary fibrosis and scleroderma. Copper is an essential co-factor required for the expression of lysyl oxidase.[15] This enzyme appears to be an intrinsic protein of connective tissue that is induced at detectable levels during fibrogenesis and fibroproliferative processes.

Progressive loss of vascularity in OSMF leads to epithelial atrophy predisposing the tissues to malignant transformation.[16] Tissue ischemia has been the foundation for use of peripheral vasodialators in the management of OSMF. Pindborg in his study of 53 biopsies found atrophy of the epithelium in 71.4% of the OSMF patients.[17] Khan et al have recently proposed a mechanism for epithelial atrophy in OSMF

mediated by arecoline and copper.[18] Copper (II) in combination with arecoline, forms arecoline copper complex. Arecoline gets oxidized along with the reduction of copper (II) to Cu (I). The reduced Cu (I) in turn donates an electron to O2 resulting in formation of superoxide radical. The superoxide radical leads to cytotoxicity of epithelial cell resulting in epithelial atrophy.

Trace metals such as copper may play an important role in the development and progression of neoplasia.[19] 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.[20] The exact mechanism of copper-induced mutagenesis is not fully understood. Copper-induced DNA damage has been reported[21] and there is evidence to suggest that copper may bind to the protein product of p53, resulting in alteration of its conformation.[22,23]

Copper in OSMF patients

Salivary copper is found to be higher in areca nut chewers. This finding indicates that soluble copper found in areca nut is released into the oral environment and its buccal absorption may contribute to fibrosis of oral tissues where copper is deposited. This observation suggested a possible local effect of copper in the etio pathogenesis of OSF.[24] Salivary copper levels appear to vary from mild OSF to severe cases.[25]

Several studies have shown that serum copper levels are higher in OSMF patients and it increases as the clinical staging of OSMF progresses raised suggesting a systemic effect of copper.[26-29] Also, the serum copper levels shows an increasing trend with increase in the duration of areca nut chewing habit.

In a cytological study, authors have shown that intense red stain was seen in OSMF smears as dark granules within the cytoplasm compared to palered staining in chewers and no staining in non-chewers.[30-31]

Significantly raised tissue copper levels in OSMF tissues has been previously demonstrated.[32] A panel of buccal mucosal biopsies from patients with OSMF was used to measure the tissue concentrations of copper by mass absorption spectrometry (MAS). By MAS, the mean tissue copper level was 5.5±2.9 mg/g in the OSF specimens compared with 4±1.9 mg/g in the non-areca chewing controls. Energy dispersive x-ray microanalysis (EDX) was used to identify the presence and distribution of the metal element. EDX showed distinct peaks corresponding to copper in the epithelium and in the connective tissue of the OSMF specimens compared to spectra obtained from control oral biopsies from non-areca chewing subjects. These findings were confirmed by secondary ion mass spectrometry (SIMS) analysis in a small number of samples.

Copper as outcome measurement in clinical trials

Keeping in view the above facts, it is suggested that including serum/salivary copper levels as a secondary outcome in the future randomized controlled trials assessing the efficacy of medicinal interventions in OSMF would be an interesting idea. The changes in the serum copper levels may be correlated to improvement in primary outcomes like maximal mouth opening and tongue protrusion. It would be more useful to evaluate serum/salivary copper levels for those medicinal interventions which affect epithelial atrophy such as peripheral vasodialators like pentoxifyllinetherapy.[33] An increase in vascularity of the tissues, may result in improvement in epithelial atrophy which may also be reflected by decrease in serum copper levels.

Conclusion

Copper has an important role in pathogenesis of OSMF. Including serum copper levels as an outcome in clinical trials assessing medicinal interventions is an attractive concept. It is prudent to include laboratory investigations in future clinical trials especially those designed to evaluate a medical intervention to improve the quality of evidence generated from such trials.

Conflict of interest: None

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