REVIEW ARTICLE

POTENTIALLY SIGNIFICANT BIOMARKERS IN ORAL SUBMUCOUS FIBROSIS

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ABSTRACT

Oral submucous fibrosis (OSMF) is a chronic, irreversibly progressive and precancerous disease that effects oral, oropharyngeal and oesophageal mucosa. OSMF is characterised by the inflamation and progressive fibrosis of lamina propria that leads to difficulty in mastication, speech, swallowing and causes limited mouth opening. Arecoline (major alkaloid) in areca nut is the main aeitiological factor in causing the disease. Prevalance of OSMF ranges from 0.2 % to 0.5% in South India. The malignant rate of transformation over 17-year period was 7.6%. Trace elements (part of metalloenzymes) are recognised as versatile biomarkers which may be helpful in early detection, prognosis and can reduce the incidence of cancer. Copper, Iron, Zinc, Selenium, Cadmium, antioxidants (Superoxide Dimutase, Vitamin A, Vitamin C, Vitamin E), immunoglobulins and alternation in oncosupressor genes and other genes have been emphasized as biochemical parameters that play an important role in its pathogenesis. These parameters can also serve as important biomarkers in early detection of a premalignant condition and cancer progression.

Key Words : Oral submucous fibrosis, trace elements, biomarkers, antioxidants, oncosuppressor genes

INTRODUCTION

Oral submucous fibrosis is a precancerous disease with immuno-imflamatory processes and if persistent, will result in activation oncogenes and loss of tumor suppressor genes that will promote abnormal cell growth and risk of cancer [1]. It was first described as ' Atrophica idiopathic mucosa oris' by Schwartz (1952) in five Indian women in Kenya as a fibrosing condition. Later it was characterised as insidous, chronic disease by Pindborg (1966) in any part of oral cavity and pharynx [2]. Oral submucous fibrosis is now recognised as an Indian disease all over the world and has the highest potential of malignancy among all other premalignant lesions [4]. The malignant rate of transformation over 17-year period was recorded at 7.6 Prevalence of OSMF was approximately 0.2% to 0.5% (mostly among 20 and 40 years of age) with higher percentage in South India [4].

Tobacco (smoked/chewable), pan masala, chilli, malnutrition, autoimmunity and genetic predisposition are multiple factors but areca nut has the main contribution in causation of disease[4]. Studies show that fourth most addictive substance in the world is areca nut [6]. The commercially prepared forms of areca nut are scented supari, gutka, mawa, mainpuri tobacco, pan masala and betel quid (may be either with or without tobbaco) in the presence of slaked lime, catechu, flavouring agent, etc. These factors play the key role in phenotypical alteration of fibroblasts which lead to fibrosis of oral mucosa [5]. Clinically, patients with such fibrotic disease suffers from sensitivity to spicy food, vesiculations, ulceration, blanch-ing, stiffness of oral mucosa resulting in the rigidity of tongue and trismus. Histological findings show focal parakeratosis or hyperkeratosis and atrophy of oral epithe-lium [7].

Trace elements (part of metalloenzymes) are recognised as versatile biomarkers that may be helpful in early detec-tion, prognosis and can reduce the incidence of oral cancer [8]. Biochemical investigations of blood, serum and tissues are the best indicators for disease progress and may serve in broad spectrum analysis of causation of potentially malignant condition [9].

The molecular basis for the changes caused by Arecanut Insert the flow chart along with explanation

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MATERIALS AND METHOD

This review included all articles that were used for the advancment of information about potential biomarkers in oral submucous fibrosis. Appropriate articles were determined according to a reconsideration of abstracts. Search of academic and published literature was carried utilizing the electronic databases of Pub Med, Google scholar, Elsevier from 2000 to 2014 for English-language articles. The research terms applied were: "biochemical markers and Oral Submucous Fibrosis", "Biomarkers of OSMF"and "OSMF". The subjects, titles and abstracts of articles were appraised. Entire and complete text matter and reviews of the studies and researches were analyzed when the abstract corresponding to the inclusion framework. Evaluation of selected data include a serious and detailed review of abstracts or full text papers.

Trace elements

Copper

Copper is the nutrient essential to carry out enzymatic functions important for human metabolism, including cytochrome-c oxidase, superoxide dimutase, metallothene and lysyl oxidase. In vitro, raised copper concentrations show increase proliferation of fibroblasts. High copper and ceruloplasmin levels were observed in patients with pre-malignant and malignant oral lesions[4]. Areca nut has a high copper content (302 nmol/g) , the substantial amount of which is released into saliva[12] after 15-30 minutes of chewing areca nut [13].

Some studies show that high serum copper is responsible for the severity of OSMF [12]. Margalith et al investigated that damage by copper ions is due to superoxide radicals. These complexes react with hydrogen peroxide to form hydroxyl radicals that causes destruction of RNA, DNA and protein ultimately resulting in the malignancy[14]. The reason behind increase serum copper might be the realease of copper containing the ceruloplasmin due to inflamatory response by liver or reduced degradation of serum ceruloplasmin. Cytological study confirmed the important role of copper in pathogenesis of OSMF showing intense staining in smears of OSMF as compared to smears from non chewers. Thus, it can prove an efficient marker of early diagnosis of malignant transformation [15].

Iron

Epidemiologic studies have established the role of diets rich in vegetables and fruits in oral carcinogenesis, with important conribution of vitamins and iron in maintainance of oral mucosa ([16]). Iron plays an important role in development, maintenance and defense abilities of oral mucosa. It effects the ability of iron containing enzymes which require heme, biological oxidations, transport and is necessary for DNA, RNA, collagen and antibody synthesis ([9, 17]). Anemia can be treated by increase iron intake, either by diet diversification, supplementation, or fortification of foods. The best long-term approach in reduction of the incidence of iron deficiency is food fortification [38].

Oral submucous fibrosis is also considered as an "Asian version of Sideropenic dysphagia". Chronic iron deficiency leads to mucosal exposure to irritants such as arecanut [18]. Peptidyl lysine hydroxylase requires molecular oxygen, ferous iron, alpha-ketoglutrate and ascorbic acid to form collagen type I coupled with loss of more soluble collagen type III and type IV[14,18] In eastern countries, Oral submucous fibrosis (OSMF) may be the manifestation of chronic iron (Fe) deficiency and a counterpart of Plummer-Vinson syndrome. The reasons of iron deficiency in OSMF patients could be multifactorial. It may be due to its utilization in collagen formation by the process of hydroxylation of proline and lysine, altered epithelial cell turnover rate or depletion of nutrients due to pronounced difficulty in mastication [19].

Zinc

Zinc (Zn) is an important part of biomembranes that manages membrane stability and lipid peroxidation-related injury. It has a role in RNA and DNA polymerase, inhibitory effect on phosphodiesterase, activation of membrane-bound adenylcyclase thus suggesting a role of zinc in carcinogenesis. . Zinc deficiency also contributes to cancer initiation by activation of NF-kB expression and the consequent induction of tumorigenic signaling [20].

Selenium

Selenium (antioxidant nutrient) has been considered as an integral part of the glutathione peroxidase enzyme, type 1 iodothyronine deiodinase, metalloprotein, fatty acid binding protein and selenoprotein P [9, 24]. Low serum, plasma or blood levels of selenium have been found to be associated with the incidence of malignant lesions of the oral cavity[24,25], breast[26,27], ovary[25,28,29], oesophagus[24, 25], colon[28, 29] and prostate [28, 29]. It is responsible for immune modulation and cells growth inhibitory properties that effects immune response by making immune cells more resistant to oxidative stress [24, 30]. According to the various epidemiological studies, selenium is considered as a protective agent and its dietary intake is of great benefit against cancer [30].

Cadmium

Rajendran et al [21] noticed elevation of cadmium (Cd) levels in OSMF where as cadmium values were decreased in Oral Cancer and oral leukoplakia. Cadmium accumlates in the body hence cadmiumn burden increases with age. Increase intestinal absorbtion of Cd (a component of ghutka) can be linked to low iron status in OSMF patients. The Cadmium burden in the body will replace zinc (anti cariogenic agent) and will show its cariogenic effect by reducing zinc anticancer activity [21]. Cadmium may be one of the cause for malignant transformation of OSMF and its estimation may be a helpful tool in differential diagnosis of premalignant and malignant lesions of the oral mucosa.

Antioxidants

Reactive oxygen species (ROS) generation initiates lipid peroxidation (LPO) which highly promotes the carcinogenesis process. Antioxidants especially enzymatic antioxidant like Superoxide Dimutase (SOD), beta carotene and Vitamin A, Vitamin E, Vitamin C and Vitamin E play an important role in this process [9]. Stahelin et al in his 12-year research on vitamins, plasma antioxidants and subsequent cancer mortality proved that decrease levels of antioxidants such as beta carotene, Vitamin C and Vitamin E is linked with the increased mortality rate due to cancer [31].

Superoxide Dismutase
Betel quid generates free radicals in the oral cavity. It is initiated by lipid peroxidation while enzymatic antioxidant superoxide dismutase detoxifies the effect of these harmful radicals (hydrogen peroxide and hydroxyl). These radicals transfer their unpaired electron to oxygen to form superoxide in order to prevent oxidative stress [14, 34]. Beta carotene and Vitamin A

Beta carotene (red-orange coloured pigment) is abundantly present in plants and animals. It is the inactive precursor of Vitamin A [9]. Beta carotene ingestion quickly increases helper T lymphocytes. It plays an important role in OSMF and its level decreases with disease progression [32]. An irreversibly oxidised form of Vitamin A is retinoic acid which is the principal hormone-like growth factor for maintainance of epithelial and other cells (9). It has immuno-regulatory properties and an excellent radical trap for hydroxyl and peroxyl radicals, therefore it should be maintained in adequate levels in the blood [32].

Vitamin C (Ascorbic acid)
Vitamin C is an antioxidant scavenging free radical,

reduces vitamin E degradation, inhibits nitrosamine formation, enhances detoxification via cytochrome P450 and iron absorption by reducing dietary iron from ferric form to the ferrous form. Vitamin C is utilized in conversion of proline into hydroxyproline. This hydroxylation reaction requires ferrous iron and Vitamin C. Lysyl oxidase upregulates the collagen cross linkages in the presence of Vitamin C that results in the advancement of the condition from the stage I to stage II. Rajendran et al proved that deficiency of vitamins and iron will result in abnormal repair of the lamina propria. This will result in defective healing and scar formation, which ultimately led to OSMF. Singh et al concluded that the therapeutic supplementation of Vitamin C reduces the oedema between the collagen bundles and regenerates new collagen bundles with good approximation in OSMF patients [33].

Vitamin E
Vitamin E is the fat soluble antioxidant that include both tocopherols and tocotrienols. It ceases the production of reactive oxygen radicals (ROS) formed after the oxidation of fat [9]. Gupta et al evaluated antioxidant parameters and found decreased Vit E level in stage II and III but not in stage I OSMF patients. Studies show that OSMF or the products associated (areca nut and additives) induce oxidative stress on the tissues [9, 35, 36].

Immunoglobins

The role of active immune response in OSMF is to accelerate body protection and detection of the foreign antigen. This process will cause abnormal lymphocyte function and hyperactivity of B cells. High levels of IgG were observed among OSMF patients by Gupta et al, which is one of the earliest-recorded studies in India [14]. In few recent studies increase in IgG and IgA has been noted in OSMF patients. [14, 37].

Alterations in Oncosuppressor Genes and Other Genes
It was evident from earlier studies, that some oncosuppressor genes play an important role in areca related carcinogenesis [39, 40, 41]. A progressive reduction of PTEN expression (tumor suppressor gene) was noticed in Oral submucous fibrosis (OSMF) and Oral Squamous Cell Carcinoma (OSCC). Hence, PTEN alteration is considered as a specific molecular event in carcinogenesis [41, 42, 43]. FHIT (Fragile Histidine Triad) is expressed in the epithelium of normal oral mucosa and a decrease in the expression of FHIT was noticed in OSMF and more significantly in carcinoma arising from OSMF. There were 716 genes upregulated and 149 genes downregulated among OSMF patients identified through oligonucleotide microarray that are responsible for pathogenesis and malignant transformation of OSMF [41]. Genomic instability denotes early genetic events during the malignant transformation of the disease. This may be due to the Loss of Heterozygosi-ty (LOH) and chromosomal copy number abnormality [41, 42].

The cytochrome P450 (CYP) gene family functions actively in oxidative metabolism of active endogenous and xenobiotic substrates. Cytochrome P450 has been identified as a genetic biomarker for susceptibility to OSMF and authors have further suggested that individuals with high genetic risk for OSMF could be investigated according to the genetic polymorphisms in some exclusive regions of the Cytochrome P450 3A genes [46]. Genes like CYP2B6, CYP2C18, CYP2F1, CYP3A5, microsomal glutathione

S-transferase 2 (MGST2), alcohol dehydrogenase (ADH), UDP glucuronosyl transferase 2B15 (UGT2B15), ADH1C) related to the pathway of CYP metabolism were found to be down regulated in all stages of OSMF [47]. It is suggest-ed that these polymorphisms can be the cause of high risk of OSMF among men if they use arecanut or smokeless tobacco in abundance [41, 48].

CONCLUSION

Oral submucous fibrosis has a high incidence and carries a significant morbidity rate due to its progression to oral cancer. Ceasation of areca nut and ghutka products should be the first step among such patients. Intervention studies and public health awareness programmes linked with hazards of carcinogenic products (areca nut and ghutka that has become the common trend in the Asian society), OSMF conditions and habits may prove the best way to control the disease process at community level. The evaluation of trace elements put the clinician in a better position to determine the stage of precancerous condition and also highlights the importance of iron supplementation and healthy diet as a part of overall treatment of this disease.

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Fig 1: Molecular Changes Caused By Arecanut – Salivary Arecoline concentration during betel quid chewing may lead to 5.7µg/ml- 97.4 7µg/ml. Studies show arecoline causes upregulation of cytokines (IL-6, TNF, INF alpha), Cystatin C (2), Nuclear factor-kappa B (NF-Kb), Tissue Inhibitor Metalloprotinase (TIMP) which are one of the factors responsible for increase collagen formation and MMP-2 and MMP-9 were found in minimal amounts that ultimately leads to fibrosis. Epithelial Mesenchyme Transition (EMT) a process that contributes to tumour cell invasion is a newly forwarded concept that has gained substantial attention recently.

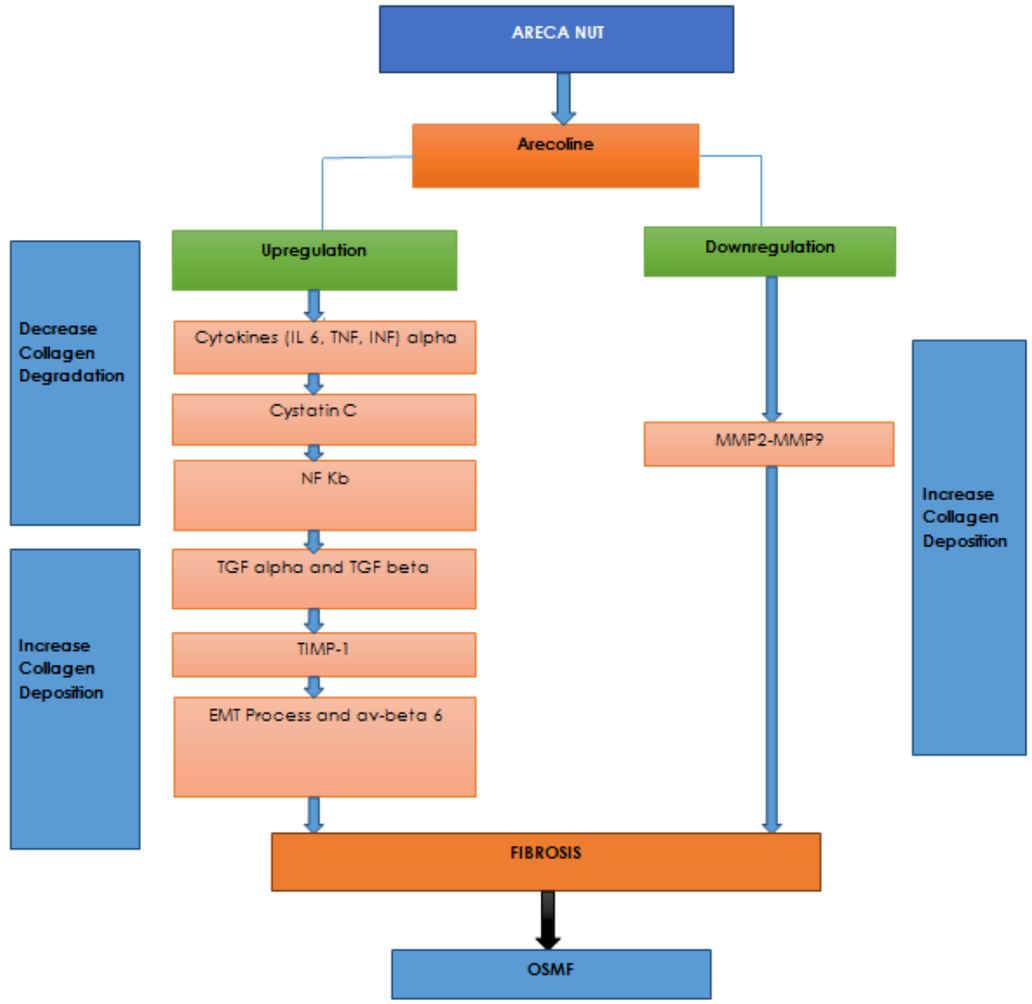

Table 1. Classification of Potential Biomarkers in OSMF

Potential Biomarkers	Role In Oral submucous fibrosis (OSMF)
1) Trace Elements	
Copper (Cu)	Forms hydroxyl radicals and increase fibroblast proliferation.
Iron (Fe)	Peptidyl lysine requires ferous iron to form collagen type 1that promotes fibrosis.
Zinc (Zn)	Active component of antioxidant enzymes (superoxide dimutase, etc), interfere in Cu absorption and acts as antifibrotic agent.
Selenium (Se)	Immune modulation and consists of growth inhibitory properties.
Cadmium (Cd)	Cd burden replace Zn (anticariogenic agent) and linked to low iron status in OSMF patients.
2) Antioxidants	
Superoxide Dimutase	Detoxifies free radical (hydrogen per oxide and hydroxyl effect.
Beta carotene and Vitamin A	Excellent radical trap and decrease disease progression.
Vitamin C (Ascorbic acid)	Upregulates collagen cross linkages, enhances detoxification and iron absorption.
Vitamin E	Ceases oxidative stress.
3) Immunoglobins	
IgG and IgA	Accelerates body protection.
4) Oncosuppressor gene and other genes alterations	
PTEN	Decrease expression in areca related carcinogenesis
FHIT	Decrease expression specifically in carcinoma arising from OSMF.
CYP gene family	Function in oxidative metabolism of active xenobiotic and endogenous substrates.

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