Diet and Micronutrients

Madhura Murittige Gopalakrishna and Roopa S. Rao

Contents

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10.1 Introduction

Oral submucous fibrosis (OSF) is a potentially malignant disorder, primarily caused by areca nut consumption, and is characterized by fibrotic changes of oral and pharyngeal tissues. The etiopathogenesis of OSF is a complex orchestration involving a multitude of molecules and enzymes [1–6]. In this chapter, we discuss the role of diet, nutrition, and micronutrients as risk or protective factors in the causation of OSF.

> WCRF/AICR defines "**Nutrition** as the set of integrated processes by which cells, tissues, organs, and indeed a whole organism acquire the energy and nutrients needed to function normally and have a normal structure" [7]

10

Nutrition plays a significant role in the growth, development, and functioning of an organism. Finally, all the energy requirements for body metabolism will be met by the nutritional status of the diet consumed. The so-called diet comprises essential nutrients that are to be mandatorily obtained from an external source, and some components will be converted into essential ingredients by the body from the consumed dietary portion. Further, the diet encompasses certain elements that are not essential nutrients but may influence the body's metabolism. Such compounds are phytochemicals, fiber, caffeine, and others [7].

> **A healthy diet includes the following: (WHO Recommendation) [8, 9]**
>
> - Fruit, vegetables, legumes (e.g., lentils and beans), nuts, and whole grains (e.g., unprocessed maize, millet, oats, wheat, and brown rice)
> - At least 400 g (i.e., five portions) of fruits and vegetables per day, excluding potatoes, sweet potatoes, cassava, and other starchy roots

A healthy diet comprises macro- and micronutrients. The **micronutrients** are needed by the body in smaller amounts, and they represent **vitamins, minerals, trace elements**, and **antioxidants**. These micronutrients have a great impact on the overall health of an individual. The micronutrients empower the body to produce hormones, enzymes, and other substances required for normal growth and development. The deficiency states of micronutrients may result in diseases affecting different parts of the body including oral tissues; further, these deficiency states may also result in fatal and life-threatening conditions [10–13].

The mineral component of the micronutrients could be further typed as macrominerals and microminerals. The microminerals are the trace elements that exist in smaller amounts in natural and perturbed environments and play a significant role in various physiological and metabolic phenomena of the human body [14].

Learning Goals

- Epidemiological evidence on diet and nutrition in OSF
- Role of trace elements (Cu, Zn, Fe, Se) in OSF
- Role of vitamins in OSF
- Evidence from interventional studies in OSF

10.2 Epidemiological Evidence on Diet and Nutrition

An epidemiological (population-based case-control) study [15], was designed to assess the dietary factors in oral potentially malignant disorders (OPMDs) in Gujarat, India. The primary objective was to check the association of dietary components (antioxidants, vitamins, minerals, and fiber) with OPMDs of the oral cavity. A food frequency questionnaire (FFQ) was developed and tested to collect the dietary information to assess the exposure to various nutrients. Out of 5018 male subjects consuming tobacco, 318 exhibited OPMDs and qualified as cases. Age- and gender-matched healthy individuals without oral lesions were selected as the control group. The common OPMDs observed were OSF and oral leukoplakia. The FFQ was composed of questions on the frequency and quantity of 92 food items consumed, reflecting >95% of exposure to comprehensive energy, fiber, fat, minerals, and vitamins. Of all the dietary elements, the fiber component was observed to be significantly protective for oral submucous fibrosis with a 10% reduction in risk per g day ($P < 0.05$). The study revealed a strong linear protective effect (OR = 0.89) on a continuous scale (g d^{-1}), $P < 0.02$.

In another epidemiological study by Gupta et al. [16], the influence of dietary factors on OPMDs in a Kerala population (India) was assessed. A customized food frequency questionnaire (FFQ) was developed and validated for estimating the nutrient exposure in the target population. In a house-to-house survey, 5056 tobacco users were screened. Among this population, 226 people exhibited OPMDs, and were recruited as cases. Equal number of age- and gender-matched controls were selected for the control group. OSF was the second common OPMD (next to oral leukoplakia) observed in the population. The confirmatory diagnosis of OSF was based on the presence of palpable fibrous bands. After adjusting for tobacco use, the intake of fruits, vegetables, and β-carotene showed an inverse relation to risk and an average reduction of about 10% per quartile of exposure. Zinc was shown to have a dose-response gradient and a larger effect in men. This study was undertaken to check the reduced risk of OPMDs in individuals who consume more fruits and vegetables; it confirmed the influence of these dietary factors in the development of OPMDs that had included both oral leukoplakia and oral submucous fibrosis [16].

The next section of this chapter highlights the role of dietary components in the disease process of OSF. Here, we discuss the role of trace elements, e.g., copper, zinc, iron, and selenium, in OSF and the role of vitamins in OSF.

10.3 Role of Trace Elements in OSF

10.3.1 Role of Copper

Copper (Cu) is the third most ample trace element in humans. Copper accounts for 75–100 mg of the total body. Copper is found almost in every tissue of the body, and the chief storage organs are the liver and brain, heart, kidney, and muscle. Further, copper is transported as ceruloplasmin into the plasma and excreted in the bile. In erythrocytes, 60% of the copper is found as copper-zinc metalloenzyme superoxide dismutase, and the remaining 40% is bound to other amino acids and proteins. Copper is a significant component of various enzymes involved in vital biological functions. Of significance in OSF is the synthesis of collagen and elastin as copper is a cofactor for the enzyme lysyl oxidase. Moreover, copper can act as an antioxidant protecting the tissues from oxidative stress, as well as a prooxidant causing damage to tissues [17–24].

The WHO Collaborating Centre research group based at King's College London first described raised copper levels in areca nut [25]. Copper dissolves in saliva and remains in the oral fluids for 30 min. This facilitates the uptake of copper by oral epithelial cells. The absorption of copper by oral mucosal keratinocytes is by a nonenzyme-dependent diffusion process, bound to metallothionein. With regard to areca nut chewing, there is raised level of copper seen in OSF patients. The higher copper levels upregulate the activity of lysyl oxidase causing more collagen production [26, 27]. In addition, when Cu is found in higher concentrations, there is a release of active oxygen species that further brings about oxidative damage to the cell [28, 29].

Several subsequent studies have shown elevated levels of Cu in the sera of OSF patients [29–34]. This has been attributed to the chewing of areca nut that is rich in Cu (302 nmol/g). The liver releases ceruloplasmin, a copper-carrying protein. The decreased catabolism of ceruloplasmin increases Cu levels in OSF patients. Further, the higher levels of Cu induce oxidative stress by Fenton and Haber-Weiss reaction. The serum copper levels in OSF patients show a gradual increase with advanced stages of the disease [30–35].

The role of Cu has been an interesting subject of investigation in carcinogenesis. High levels of copper within the cells generate hydroxyl radicals that can result in damage to the DNA and proteins. This may activate tumor necrosis factor-alpha and vascular endothelial growth factors. These factors are important for tumor growth and metastasis. During the malignant transformation of OSF, a four- to eightfold increase in the blood level of ceruloplasmin has been observed. Ceruloplasmin acts as a source of Cu ions, initiates LDL oxidation, and plays a role in the malignant transformation of OSF to carcinoma [36–42].

However, two studies have reported low levels of Cu in OSF patients when compared to healthy volunteers providing contradictory evidence. Varghese et al. speculated that the reduced Cu levels observed in the study could be attributed to the difference in laboratory methodologies employed and patient selection. Atomic absorption spectrophotometry was used to measure Cu levels, and the patients recruited for the study were not on any treatment in contrast to earlier studies where colorimeter was used and patients were on some form of treatment for OSF [43]. The study by Anuradha et al. also reported lower levels of Cu in OSF patients. In this study the cohort had poor dietary patterns and loss of appetite, suggesting reverse causation, also, the sample size was low [44]. However, many later studies have shown raised Cu levels in OSF patients, with atomic absorption spectrophotometry analysis [32, 45, 46].

10.3.2 Role of Zinc

Zinc (Zn) is the second most abundant transition metal in humans that appears in all enzyme systems. In blood plasma, Zn is transported by albumin and transferrin. Zn exhibits catalytic, regulatory, and structural roles in a biological system. Zn also shows antioxidant and antimicrobial properties.

An animal study (on rodents) has shown that Zn-deficient diet could result in change in the keratinization pattern (parakeratosis from orthokeratinization) of the oral mucosa.

Zn is a cofactor for the superoxide dismutase enzyme, and many studies have shown lower levels of Zn in OSF patients. This could be due to higher Cu levels and oxidative stress. Another interesting finding is lower levels of iron and higher serum levels of Zn in OSF patients. This is due to the common transporter molecule, transferrin, that carries both iron and Zn. Thus, OSF patients exhibiting lower iron levels would exhibit higher serum Zn levels.

The natural antioxidant of humans, superoxide dismutase, is a Cu–Zn protein complex that shows an anticarcinogenic effect in OSF. Additionally, Zn reduces the activity of Cu-coupled lysyl oxidase and thus inhibits collagenic cross-linkage. Further, Zn promotes collagenic degradation via collagenases and matrix metalloproteinases. By interfering with the mucosal absorption of Cu, Zn shows an inverse relation with Cu. Higher Zn levels hamper Cu absorption as both the metals are absorbed through metallothioneins. Thus, the Cu:Zn ratio could be a reliable biomarker for assessing carcinogenesis. There is limited literature suggesting a carcinogenic effect of Zn [47–58].

The Zn level in body fluids (serum/plasma/saliva) of OSF patients has been evaluated in numerous studies. Of these, only four studies have not reported lower Zn levels in OSF patients. In one such study by Khanna et al., higher Zn levels were observed in OSF patients, but it was not statistically significant. The reason for elevated Zn levels was attributed to consumption of gutkha with higher Zn content [31, 33, 35, 44–46, 59–65].

The various effects of reduced Zn levels in OSF patients are the following:

(i) Zn acts as a first-line defense against oxidative stress by forming cofactor Cu/Zn-superoxide dismutase enzyme [66].
(ii) Zn is pivotal for the gene expression of metallothionein, which removes hydroxyl ions and confers protection against oxidative damage [67].
(iii) Zn competes with other transition metals for binding sites, thereby reducing those metals from generating hydroxyl ions [67].
(iv) Excessive cellular uptake of Zn for neutralization of free radicals [68].

Important attributes of Zn in preventing the development of malignancy [67]:

(a) The tumor suppressor protein, p53, is Zn dependent and is involved in the repair of DNA.
(b) The apoptotic regulating transcription factors, AP-1 and NF-kB, show alterations with reduced cellular levels of Zn.

Lower levels of Zn may induce overexpression of COX-2 that promotes cell proliferation, prevents apoptosis, and therefore contributes to the malignant transformation of OSF [67].

10.3.3 Role of Iron

Iron (Fe) is the most abundant essential trace element in humans. Iron is an important component of heme and various enzymes in the body [69, 70]. The vital functions carried out by iron are the transport of oxygen, synthesis of DNA, energy metabolism, development and maintenance of oral mucosa. Iron deficiency leads to Plummer-Vinson's syndrome. Patients who have Plummer-Vinson's syndrome (sideropenic dysphagia) exhibit features of anemia; further glossitis, angular cheilitis, and koilonychia are noticed. These patients have greater risk of developing oral, postcricoidal, and esophageal carcinomas.

Further, the investigations on iron levels in OSF patients have revealed lower serum iron concentrations when compared to healthy controls [44, 60, 71–80].

The suggestions for diminished levels of iron in OSF patients could be due to:

(i) Excessive use of iron for the hydroxylation of lysine and proline during collagen synthesis.
(ii) The mechanical injury caused by areca nut chewing hampers intake of a nutritionally balanced diet.
(iii) Vegetarian diet may predispose an individual to greater depletion of iron stores.

Ultimately, chronic iron deficiency in areca nut chewers is a factor that facilitates the development of OSF. Further, features of anemia have been noticed in the advanced stage of OSF [81, 82].

10.3.4 Role of Selenium

Selenium is yet another vital trace element that is an important constituent of antioxidant enzymes: glutathione peroxidase and thioredoxin reductase [83]. Lower serum levels of selenium have been reported in OSF patients when compared to normal individuals [84].

10.4 Role of Vitamins

In a case-control study (OSF, n = 40; control, n = 25), deficiency of vitamin B12 and iron was reported in OSF patients when compared to healthy volunteers. Further, the red cell indices such as packed cell volume, mean corpuscular volume, and mean corpuscular Hb were significantly reduced in OSF patients [85].

Another case-control study showed high frequencies of vitamin B12, folic acid deficiencies, and gastric parietal cell antibody positivity in OSF patients when compared to healthy individuals [86].

Shetty et al. in their study observed lower serum and salivary levels of ascorbic acid with progressibe worsening of histopathological grading of OSF. The likely reason for lower levels of ascorbic acid is that it may have been used for the excessive synthesis of collagen during the progression of OSF [87].

Another study evaluated mean serum vitamin A and vitamin E levels in OSF patients. There was no statistically significant difference in these vitamin levels between OSF and control groups. It has been suggested that these vitamins are used by the tissues to combat oxidative stress generated due to the consumption of areca nut [88].

10.5 Interventional Studies

In an interventional study on OSF by Maher et al. [89], the beneficial clinical response to multiple micronutrients was evaluated in Karachi, Pakistan. Out of 169 OSF subjects recruited for the study, 117 compliant individuals were given daily oral micronutrient (vitamins and minerals) supplementations for 1–3 years. There was a significant improvement in symptoms, such as burning sensation, intolerance to spicy food, and restricted mouth opening. The interincisor distance increased in 48 (41%) and there was regression of concomitant lesions like oral leukoplakia and/or erythroplakia [89].

Summary

Points of clinical relevance:

Factors that promote fibrosis in Oral Submucous Fibrosis are as follows:

- Low dietary fibre
- Higher levels of copper
- Lower levels of Zinc, Iron, and Selenium
- Lower levels of Vitamins A, B, C, and E
- Dietary supplementation of the micronutrients has shown improvement in signs and symptoms of OSF

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