

Serum vitamin c and iron levels in oral submucous fibrosis

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Abstract

Background: In this study Serum Vitamin C and Iron levels in Oral submucous fibrosis (OSMF) were estimated. The objective was to evaluate the correlation between Serum Vitamin C and Iron levels in OSMF individuals. Serum Iron content can be a predictor for the progression of this condition. OSMF is basically a disorder of collagen metabolism where Vitamin C gets utilized in conversion of proline into hydroxyproline, this hydroxylation reaction requires ferrous Iron and Vitamin C. Many studies regarding micronutrients and other antioxidants levels have been emphasized, but very few studies are done on the Serum levels of Vitamin C and its correlation with Iron in OSMF patients. **Methods:** Thirty five OSMF patients and 50 deleterious habit free healthy individuals (controls) were selected. Two ml of venous blood was collected from each individual. Vitamin C level in serum was estimated by 2-4 dinitrophenylhydrazine method and Iron estimated by Tripyridyl method. **Results:** The level of Serum Vitamin-C and Iron was significantly decreased in OSMF patients when compared to controls which were statistically significant. **Conclusion:** On the basis of these observations, it seems possible that the chemical, thermal and/or mechanical factors associated with the use of areca nut may act in conjunction with the Vitamin C and Iron deficiency leading to the development of OSMF. Therapeutic substitution of vitamin C and Iron may be recommended in the management of OSMF

Key words: Iron, oral submucous fibrosis, Vitamin C


INTRODUCTION

Oral Cancer accounts approximately 40% of all cancers in the Indian subcontinent.^[1] A significant proportion of oral squamous cell carcinomas (OSCC) develop from premalignant lesions such as leukoplakia and conditions such as oral submucous fibrosis.^[2] OSMF is defined as insidious chronic disease affecting any part of oral cavity and sometimes pharynx. Although occasionally preceded by and/or associated with vesicle formation, always associated with Juxtaepithelial inflammatory reaction followed by fibro elastic change of lamina propria with epithelial atrophy leading to stiffness of oral mucosa and causing trismus and inability to eat".^[3] In recent years, OSMF has received considerable attention as a precursor to cancer and occurs in younger age group individuals.^[4] It's reported risk of malignant transformation varies from 7 to 13%.^[5]

Habit of chewing areca nut is the major etiological factor of OSMF. It's extract acts as a potent stimulator for collagen synthesis in human fibroblasts culture leading to excessive accumulation of collagen, leading to fibrosis.^[6] High levels of copper in areca nuts, a major etiological factor in OSMF plays an initiating role in stimulation of fibrinogenesis by up-regulation of lysyl oxidase and thereby causing inhibition of degradation of collagen and causing its accumulation thereby causing OSMF. The high serum copper levels may also lead to generate high levels of free radicals by metal-catalyzed Haber-Weiss reaction and this can be one of the reasons for the carcinogenesis in tobacco and areca nut users.^[7]

Antioxidants are enzymes or other organic molecules that can counteract the damaging effects of reactive oxygen species in tissues.^[8] Antioxidant nutrients such as vitamin C, beta -carotene, zinc and selenium are regularly found to reduce the risk of oral cancer and precancers.^[9] Iron is required for the functioning of numerous enzymes and it is reasonable to assume

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that variation in important biochemical markers like serum Iron and Vitamin C levels may be associated with the pathogenesis of OSMF.

Iron metabolism is important to maintain the health of oral mucosa, and many disease states, including cancers, are associated with Iron depletion.^[10] Serum Iron content can be a predictor for the progression of OSMF. There appears to be an association between Serum Iron content and oral carcinogenesis.^[11] It has been also postulated that Vitamin C and Iron is interrelated as Vitamin C plays important role in absorption of Iron from the gut.^[12] Vitamin C helps the body to absorb non-heme Iron.^[13] Vitamin C enhances Iron absorption by reducing dietary Iron from ferric form to the ferrous form. Thus, Vitamin C deficiency may reduce the availability of intracellular Iron. Vitamin C is also necessary to convert folic acid to its active metabolite, folinic acid.^[14-16] Other Protective role of Vitamin C in the prevention of carcinogenesis is summarized in Table 1.

Thus, the present study is undertaken to comprehend the association between OSMF, Vitamin C and Iron by estimating the levels serum Vitamin C and Iron in patients diagnosed with OSMF and comparing the values with that of habit free healthy subjects.

METHODS

Ethical clearance from the Institution was obtained and informed written consent was obtained from participants. Thirty five OSMF patients and 50 habit free healthy individuals (controls) were selected from the Dept of Oral Medicine and Radiology, People's University. Detailed case history of all subjects was recorded which includes history of deleterious habit, systemic diseases and undergoing antioxidant/multivitamin supplementation. Detailed clinical examination was conducted and findings were recorded.

The clinical diagnosis of oral submucous fibrosis was made by using the criteria as mentioned by Khanna and Andrade, 1995.^[17]

Inclusion criteria for study group

- Subjects with definitive habit of Areca nut/tobacco in any form and alcohol

- Subjects with clinical signs and symptoms of OSMF.

Exclusion criteria

- Subjects with any other Precancerous lesions or Conditions other than OSMF
- Subjects suffering from any systemic diseases like diabetes, cardiac diseases, renal diseases, liver diseases and other malignancies
- Subjects who are taking Antioxidants/multivitamin preparations.

Inclusion criteria for control group

- Subjects without any clinical Oral lesions
- Subjects with no deleterious habits
- Subjects without systemic diseases.

Exclusion criteria

- Subjects with any Precancerous lesions/conditions and Oral cancer
- Subjects suffering from any systemic diseases like diabetes, cardiac diseases, renal diseases, liver diseases and other malignancies
- Subjects who are taking antioxidants/multivitamin preparations.

Collection of sample

Two ml of venous blood was obtained from median cubital vein, blood was allowed to clot at room temperature for 1 to 2 hours. The serum was separated by centrifuge machine at 3000 rpm for 10 minutes to get a clear serum sample, The serum thus obtained was pipetted using a micro pipette and transferred into sterile plastic storage vial and was stored at -20oC in a dark container until assay.

Methodology

Estimation of Vitamin C done by 2-4 dinitrophenylhydrazine method. The Principle of this method is that Dehydro ascorbic acid was coupled with 2,4 dinitrophenylhydrazine and the resulting derivative is treated with sulphuric acid to produce a newly observed color which is measured at 545 nm.

Iron estimation was done by ramsays method and the principle of this method is that in the presence of sodium sulphate, iron reacts with 2,2' tripyridyl reagent to form a colour complex whose absorbance was read in colorimeter.

Table 1: Protective role of vitamin C

Vitamin C: As protective role in carcinogenesis

Is an antioxidant thus scavenging free radical
Reduces vitamin E degradation
It inhibits nitrosamine formation
Enhances detoxification via cytochrome P450

STATISTICS

The results were expressed as Mean \pm SD values. The means of the controls and patients were compared using Student's *t*-test. The age of patients varied from

20 yrs to 45 yrs, mean age being 32.5 yrs. The OSMF group showed male predominance with 27 males and 8 females.

RESULTS

Mean values of serum Vitamin C and Iron of control group were (1.08 ± 0.17 mg/dl) and (144.30 ± 12 µg/dl) respectively whereas in OSMF group the values were (0.4 ± 0.23 mg/dl) and (98.87 ± 18 µg/dl) respectively [Table 2]. OSMF group showed significantly lower levels of serum Vitamin C and Iron (*P* < 0.001) [Figures 1 and 2].

DISCUSSION

In the present study, decrease in Serum Vitamin C levels in OSMF group which is in accordance with the studies conducted by other authors.^[18,19] They attributed poor nutritional status^[20] and oxidative stress in precancerous lesions and conditions were responsible for decrease in serum and salivary Vitamin C and Vitamin E levels.^[19]

In OSMF patients, there is an increase in the production of highly cross linked insoluble collagen type I, loss of more soluble pro-collagen type III and collagen type VI. The cross linking of collagen due to the up-regulation of lysyl oxidase, plays a crucial role in the development and progression of the condition from stage I to stage II.^[21] Vitamin C levels decreases perhaps because of its utilization in collagen synthesis.^[7] Rajendran *et al.* who reported that Vitamins and Iron deficiency

together with malnourished state of the host leads to derangement in the inflammatory reparative response of the lamina propria with resultant defective healing and scarification which ultimately leads to OSMF.^[22] Poor nutrition is one of the causative factors of OSMF.^[23] Diet rich in high fiber and Vitamin C both has protective effect on development of OSMF and leukoplakia.^[24] In fact, case control studies have shown that consumption of carotene rich vegetables and Vitamin C rich fruits markedly reduced risk of oral cancer.^[25] Thomas *et al.* reported that high intake of fruits and vegetables can act as protective shield for OSMF.^[23] Singh *et al.* concluded that the therapeutic supplementation of the vitamin C reduces the oedema between the collagen bundles and helps in regeneration of new collagen bundles with good approximation in OSMF patients.^[26]

This study showed significant reduction in serum iron levels when compared with the values of the control group which is in accordance with the other studies reported in the literature.^[20,27] It is well-documented that patients with severe Iron deficiency condition, known as Siderophagic dysphagia or Plummer-Vinson syndrome, are at a higher risk of developing oral carcinoma, postcricoidal carcinoma and esophageal carcinoma. OSMF is also known as Asian version of 'siderophagic dysphagia'.^[28]

Occurrence of Iron deficiency is known to be present in oral cancer. Iron is also required for collagen synthesis by enzymes in hydroxylation of proline and lysine. This hydroxylation of proline and lysine is catalyzed by proline hydroxylase and peptidyl lysine hydroxylase respectively.^[29] Peptidyl proline hydroxylase requires as co-factory molecular oxygen, ferrous Iron, Alpha-ketoglutarate and Vitamin C.^[30] Therefore in OSMF individual's Serum Iron level is decreased which can be indirectly correlated with decreased Vitamin C level.

In one study where OSMF individuals were supplemented with a combination of micronutrients (Vitamins A,

Table 2: Comparison of vitamin C and iron level in serum between study and control group

Groups	Vitamin C (mg/dl)	Iron (µg/dl)
Control	1.08	144.3
OSMF	0.4	98.87

OSMF: Oral submucous fibrosis

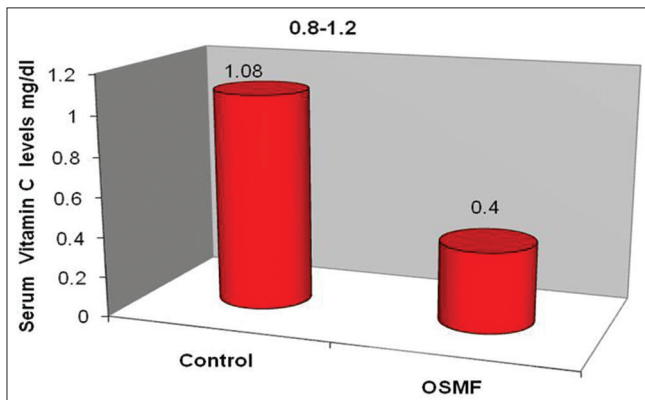


Figure 1: Level of Vitamin C in OSMF and Healthy controls

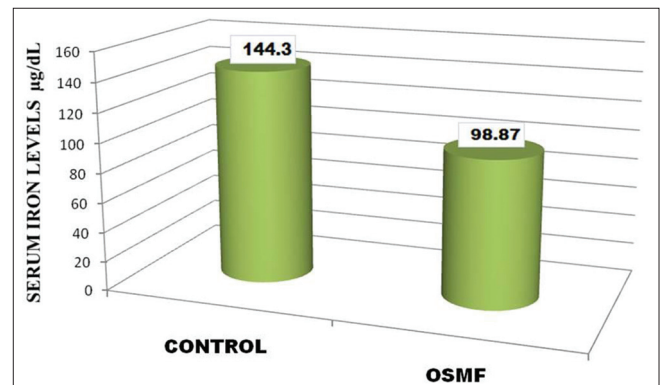


Figure 2: Level of Serum Iron in OSMF and Healthy controls

C, D, E, all eight B Vitamins, calcium, magnesium, Iron, phosphorus, copper, manganese, zinc, and molybdenum) there was a significant improvement in symptoms, especially intolerance to spicy food, burning sensation, and difficulty in mouth opening. The results indicated that supplementation with multiple micronutrients including Vitamin C and Iron can produce a clinical response in patients with OSMF.^[26]

Cytochrome oxidase, an Iron dependent enzyme, is required for the normal maturation of the epithelium. Deficiency of Iron in tissues causes epithelial atrophy due to 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 scarification. Thus, the cumulative effect of these initiating and promoting factors leads to further fibrosis, which is a hallmark of OSMF.^[31]

CONCLUSION

From the present study, it is evident that by estimation of Serum Vitamin C levels in OSMF patients, one can assess the degree of oxidative damage resulted by this disease.

Further correcting the underlying deficiency of Vitamin C along with Iron supplements improves treatment thereby arresting it in early stages and avoiding the possible consequences of malignant transformation. However further elaborate studies with a larger sample size including OSMF with coexisting oral cancer along with follow-up are needed to ascertain the actual role of these parameter in the initiation and promotion of carcinogenesis.

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