

# Evaluation of the Correlation between Serum Vitamin C and Iron Levels in Individuals with Oral Submucous Fibrosis (OSMF)

Nishu Vakil<sup>1</sup>, Surinder Sachdeva<sup>2</sup>, Jyotsna Goyal<sup>3</sup>, Balbir Kaur<sup>4</sup>

<sup>1</sup>Dental Surgeon, Department of Periodontology, Indira Gandhi Government Dental College, Jammu, India

<sup>2</sup>Professor and Head, Department of Periodontology and Implantology, MMCDSSR, MMU, Mullana, Ambala, India

<sup>3</sup>Reader, Department of Periodontology, Luxmi Bai Institute of Dental Sciences and Hospital, Patiala, India

<sup>4</sup>Professor and Head, Department of Forensic Medicine, NMCTH, Biratnagar, Nepal

**Abstract:** *Background:* In recent years, OSMF has received considerable attention as a precursor to cancer and occurs in younger age group individuals. Oral submucous fibrosis (OSF) is a potentially malignant disorder that causes fibrosis and inflammation of the oral mucosa. *Aim:* To study the correlation between Serum Vitamin C and Iron levels in OSMF individuals. *Methods:* This prospective study was conducted on forty patients seeking treatment for Oral submucous fibrosis. Same number of healthy individuals (controls) were also selected. Detailed history of all study subjects was recorded. Levels of Vitamin C and Iron were estimated in venous blood by standard methods. *Results:* OSMF group showed significantly lower levels of serum Vitamin C and Iron. Mean values of serum Vitamin C and Iron of control group were (1.06 ± 0.12 mg/dl) and (148.24 ± 18 µg/dl) respectively whereas in OSMF group the values were (0.3 ± 0.20 mg/dl) and (101.06 ± 15 µg/dl) respectively. *Conclusion:* Various factors associated with the use of areca nut along with the Vitamin C and Iron deficiency may act as risk for developing Oral submucous fibrosis.

**Keywords:** Oral submucous fibrosis, Vitamin C, Iron, correlation

## 1. Introduction

Oral submucous fibrosis (OSF) is a potentially malignant disorder that causes fibrosis and inflammation of the oral mucosa. The prevalence of OSF cases has increased from 0.03% to 6.42% in the last four decades, making it a significant public health problem in India.<sup>1</sup> A significant proportion of oral squamous cell carcinomas develop from premalignant lesions such as leukoplakia and conditions such as oral submucous fibrosis.<sup>2</sup> Habit of chewing areca nut is the major etiological factor of OSMF.

In recent years, OSMF has received considerable attention as a precursor to cancer and occurs in younger age group individuals.<sup>3</sup> The condition begins as an inflammatory response to areca nut chewing, followed by fibrosis of the oral submucosa and then progresses to induce restriction of the mouth opening and difficulty in mastication and swallowing.<sup>4</sup>

Altered trace element status has been reported in both the potentially malignant and malignant stages. Antioxidant nutrients such as vitamin C, beta-carotene, zinc and selenium are regularly found to reduce the risk of oral cancer and precancers.<sup>5</sup> Excessive collagen synthesis in OSF may result in a decline in the levels of serum and plasma Fe in OSF patients. The burning sensation and the restricted mouth opening in this disease limits the intake of food, further exacerbating deficiencies of micronutrients.<sup>6</sup> Thus, this study was planned to evaluate the correlation between Serum Vitamin C and Iron levels in OSMF individuals.

## 2. Methods

The study was conducted at a tertiary care teaching dental hospital of northern India. This prospective study was conducted on forty patients seeking treatment for Oral submucous fibrosis. The clinical diagnosis of oral submucous fibrosis was made by using the criteria as per Khanna JN, Andrade NN., 1995.<sup>7</sup> Same number of healthy individuals (controls) were also selected.

Detailed history of all study 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.

**For study group:** *Inclusion criteria* were- Subjects with definitive habit of Areca nut/tobacco in any form and alcohol; and Subjects with clinical signs and symptoms of OSMF. *Exclusion criteria* were- 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.

**For control group:** *Inclusion criteria* were- Subjects without any clinical Oral lesions; Subjects with no deleterious habits; Subjects without systemic diseases. *Exclusion criteria* were- 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.

Two ml of venous blood was collected, blood was allowed to clot at room temperature for 1 to 2 hours. The serum was separated by centrifuge machine at 3000 rpm for 15 minutes to get a clear serum sample, The serum was pipetted using a micro pipette and transferred into sterile plastic storage vial and was stored at -20°C in a dark container until assay.

Levels of Vitamin C was estimated done by 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.

Written and informed consent was obtained from study subjects. Permission of ethical committee was obtained from the Institutional Ethics Committee. All the questionnaires were manually checked and edited for completeness and consistency and were then coded for computer entry. After compilation of collected data, analysis was done using Statistical Package for Social Sciences (SPSS), version 21 (IBM, Chicago, USA). The results were expressed using appropriate statistical variables.

### 3. Results

OSMF group showed significantly lower levels of serum Vitamin C and Iron. Mean values of serum Vitamin C and Iron of control group were (1.06 ± 0.12 mg/dl) and (148.24 ± 18 µg/dl) respectively whereas in OSMF group the values were (0.3 ± 0.20 mg/dl) and (101.06 ± 15 µg/dl) respectively. (Table 1) This finding was statistically significant.

**Table 1:** Serum Vitamin C and iron levels between study and control group

Study group	Vitamin C (mg/dl)		Iron (µg/dl)	
	Mean	SD	Mean	SD
Oral submucous fibrosis	0.3	0.20	101.06	15
Control	1.06	0.12	148.24	18

### 4. Discussion

One of the major etiological factor behind OSMF is habit of chewing areca nut as also observed in this study. The areca nut is composed of alkaloid components.<sup>8</sup> The most potent alkaloid, arecoline, causes an abnormal increase in the production of collagen by the oral mucosal fibroblasts. This over production leads to the development of fibrotic bands in the buccal mucosa of OSF patients.<sup>9</sup> The condition begins as an inflammatory response to areca nut chewing, followed by fibrosis of the oral submucosa and then progresses to induce restriction of the mouth opening and difficulty in mastication and swallowing. The early stage symptoms include burning sensation in the mouth, development of ulcers and vesicles, increased salivation, and blanching of

the mucosa. In the later stages, the mucosa becomes inelastic and leathery because of the fibrotic bands.<sup>10</sup>

Altered trace element status has been reported in both the potentially malignant and malignant stages.<sup>1,11</sup> Microminerals are well established to be essential in metabolism as components of enzymes and hormones in the body. This research will focus on three trace minerals—Zn, Cu, and Fe—that are altered in OSF. Zinc is crucial for the normal functioning of the immune cells, antioxidant defense, wound healing, and stability of biological membranes.<sup>12</sup> Chewing of areca nut generates reactive oxygen species, which can cause damage to the proteins and nucleic acids in the body.

Zn induces activation of antioxidant enzyme superoxide dismutase which inhibits production of reactive oxygen species. The levels of SOD were found to be reduced (75.42 ± 7.04) in OSF patients as compared to controls (177.17 ± 5.92).<sup>13</sup> The mineral copper plays an important role in the formation of red blood cells and synthesis of collagen in the bones and connective tissue and aids in the absorption of iron. It is also essential for the functioning of enzymes such as Cu/Zn- superoxide dismutase and lysyl oxidase.<sup>14</sup> Related to OSF, Cu is a cofactor for lysyl oxidase, the enzyme involved in the maturation of collagen. The areca nut contains a high content of Cu that is released within 5–30 minutes of chewing the nut, inducing upregulation of lysyl oxidase in the oral mucosa. Trivedy et al. observed that the levels of Cu in the saliva peaked after 10 mins in two volunteers and 20 minutes in the third volunteer after chewing an areca nut product for 30 minutes.<sup>15</sup> It has been suggested that the elevated levels may then induce excessive collagen formation by the fibroblasts.

Iron is required for the functioning of numerous enzymes such as cytochrome oxidase, xanthine oxidases, succinate dehydrogenase, glucose-6-phosphate dehydrogenase, catalases, peroxidases, and choline dehydrogenase. It acts as a cofactor for prolyl hydroxylase (PH) and lysyl hydroxylase, which are enzymes involved in hydroxylation of collagen. Excessive collagen synthesis in OSF may result in a decline in the levels of serum and plasma Fe in OSF patients. Finally, the burning sensation and the restricted mouth opening in this disease limits the intake of food, further exacerbating deficiencies of micro-nutrients. The Fe dependent enzyme, cytochrome oxidase, is required for the development of the epithelium. A deficiency of iron in OSF reduces the levels of cy-tochrome oxidase, resulting in atrophy of the epithelium. Investigations have reported diminished Zn and Fe values in OSF subjects, as compared to healthy controls.<sup>16</sup> In contrast, Cu levels in serum/plasma/saliva have been observed to be elevated rather consistently.<sup>17</sup>

We observed that, decrease in Serum Vitamin C levels in OSMF group which is in accordance with the studies conducted by other authors. Diet rich in high fiber and Vitamin C both has protective effect on development of OSMF and leukoplakia.<sup>18</sup> Case control studies have shown that consumption of carotene rich vegetables and Vitamin C rich fruits markedly reduced risk of oral cancer.<sup>19</sup> Another study from Gujrat reported that high intake of fruits and

vegetables can act as protective shield for OSMF.<sup>20</sup> Another author from Pakistan 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.<sup>21</sup>

## 5. Conclusion

On the basis of findings of this study, it can be stated that various factors associated with the use of areca nut along with the Vitamin C and Iron deficiency may act as risk for developing Oral submucous fibrosis. Further larger controlled trials are warranted to support our findings.

## References

- [1] World Health Organization, IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, International Agency for Research on Cancer. Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines. IARC; 2004.
- [2] Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation rate in oral submucous fibrosis over a 17-year period. *Community dentistry and oral epidemiology*. 1985 Dec;13(6):340-1.
- [3] Yang YH, Lee HY, Tung S, Shieh TY. Epidemiological survey of oral submucous fibrosis and leukoplakia in aborigines of Taiwan. *Journal of Oral Pathology & Medicine*. 2001 Apr;30(4):213-9.
- [4] Bhattacharya PT, Misra SR, Hussain M. Nutritional aspects of essential trace elements in oral health and disease: an extensive review. *Scientifica*. 2016;2016.
- [5] Thakur N. Effectiveness of micronutrients and physiotherapy in the management of oral submucous fibrosis. *International Journal of Contemporary Dentistry*. 2011 Feb 20;2(1).
- [6] Jayadeep A, Raveendran Pillai K, Kannan S, Nalinakumari KR, Mathew B, Krishnan Nair M, et al. Serum levels of copper, zinc, iron and ceruloplasmin in oral leukoplakia and squamous cell carcinoma. *J Exp Clin Cancer Res* 1997;16:295-300.
- [7] Khanna JN, Andrade NN. Oral submucous fibrosis: A new concept in surgical management - Report of 100 cases. *Int J Oral Maxillofac Surg* 1995;24:433-9.
- [8] Prabhu RV, Prabhu V, Chatra L, Shenai P, Suvarna N, Dandekeri S. Areca nut and its role in oral submucous fibrosis. *Journal of clinical and experimental dentistry*. 2014 Dec;6(5):e569.
- [9] Van Wyk CW, Seedat HA, Phillips VM. Collagen in submucous fibrosis: an electron-microscopic study. *Journal of Oral Pathology & Medicine*. 1990 Apr;19(4):182-7.
- [10] Sinor PN, Gupta PC, Murti PR, Bhonsle RB, Daftary DK, Mehta FS, Pindborg JJ. A case-control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. *Journal of oral pathology & medicine*. 1990 Feb;19(2):94-8.
- [11] Humayun S, Prasad VR. Expression of p53 protein and ki-67 antigen in oral premalignant lesions and oral squamous cell carcinomas: An immunohistochemical study. *Natl J Maxillofac Surg* 2011;2:38-46.
- [12] Gupta MK, Mhaske S, Ragavendra, imtiyaz. Oral Submucous Fibrosis- Current Concept in Etiopathogenesis. *People's J Sci Res* 2008;1:39-44.
- [13] Uikey AK, Hazarey VK, Vaidhya SM. Estimation of serum antioxidant enzymes superoxide dismutase and glutathione peroxidase in oral submucous fibrosis: A biochemical study. *Journal of Oral and Maxillofacial Pathology*. 2003 Jul 1;7(2):44.
- [14] Tainer JA, Getzoff ED, Richardson JS, Richardson DC. Structure and mechanism of copper, zinc superoxide dismutase. *Nature*. 1983 Nov;306(5940):284.
- [15] Trivedy C, Baldwin D, Warnakulasuriya S, Johnson N, Peters T. Copper content in Areca catechu (betel nut) products and oral submucous fibrosis. *The Lancet*. 1997 May 17;349(9063):1447.
- [16] Shetty SR, Babu S, Kumari S, Shetty P, Hegde S, Karikal A. Status of trace elements in saliva of oral precancer and oral cancer patients. *Journal of cancer research and therapeutics*. 2015 Jan 1;11(1):146.
- [17] Okade AR, Hallikeri KS, Trivedi DJ. Salivary estimation of copper, iron, zinc and manganese in oral submucous fibrosis patients: A case-control study. *Clinical Cancer Investigation Journal*. 2015 May 1;4(3):302.
- [18] Marshall J, Graham S, Mettlin C, Shedd D, Swanson M. Diet in epidemiology of oral cancer. *Nutr Cancer* 1982;3:145-9.
- [19] Singh N, Singh J, Singh U. Oral submucous fibrosis: A new approach to treatment with combined therapy. *J Indian Dent Assoc* 1996;67:168-70.
- [20] Gupta PC, Herbert JR, Bhonsle RB, Sinor PN, Mehta H, Mehta FS. Dietary factors in oral lichen planus and OSMF in a population based study in Gujrat India. *Oral Dis* 1998;4:200-6.
- [21] Maher R, Aga P, Johnson NW, Sankaranarayanan R, Warnakulasuriya S. Evaluation of multiple micronutrient supplementations in the management of oral submucous fibrosis in Karachi, Pakistan. *Nutr Cancer* 1997;27:41-7.