**Int. j. adv. multidisc. res. stud.** 2023; 3(3):989-992

**Received:** 05-05-2023
**Accepted:** 15-06-2023

International Journal of Advanced Multidisciplinary Research and Studies

**ISSN: 2583-049X**

# Increase Serum Copper Level in Betel nut Chewer Oral Submucous Fibrosis Patient

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**Abstract**

Oral submucous fibrosis (OSMF) is a precancerous condition of the oral cavity and categorized as potentially malignant disorder. Trace elements such as Copper, Iron, and Zinc play an important role in growth and maintaining the integrity of cells and tissues related to oral mucosa. The aim of the present study was to examine the Serum Cu level in OSMF patients. This case-control study was carried out on 10 patients. OSMF patients were categorized by clinical and histopathological presentations and clinical staging. Flame Atomic Absorption Spectrometry was used to measured Serum copper level. Mean serum copper concentration was greater in study group compared to control group. The difference between Serum copper levels was statistically significant. Copper level was increased in OSMF patients and a gradual rise in copper level was noted with the advancement of clinical staging of OSMF who has exclusive history of betel nut chewing.

**Keywords:** Serum Copper, Oral Sub Mucous Fibrosis, Histology, Buccal Mucosa, Betel Nut

## Introduction

Oral submucous fibrosis (OSMF) is a precancerous condition of the oral cavity and categorized as potentially malignant disorder, affecting the oral and oropharyngeal mucosa. The dangerous result of this disease may include fibrosis, trismus, atrophic uvula, and the highest malignant transformation rate (7%–13%) [1].

The disease is frequently seen in countries of Indian sub-continent, South-East Asia, Southern China, and Polynesia. According to World Health Organization (WHO) report, more than 5 million people are affected by OSMF worldwide and ranging up to 0.4% in the Indian rural population [2]. Kumar *et al*. showed in an epidemiological study in a rural Indian population, that the malignant transformation rate of OSMF was 7.6% (5 of 66) over a 17-year period [3].

Betel nut or areca nut has a high copper content (302 nmol/g), the substantial amount of which is released into saliva after 15-30 minutes of chewing areca nut [4]. Habit of chewing betel nut is the major etiological factor of OSMF. Its extract acts as a potent stimulator for collagen synthesis in human fibroblasts culture leading to excessive accumulation of collagen, leading to fibrosis [5, 6]. In addition, alkaloid components from areca nut such as Arecoline causes an abnormal increase in the production of collagen by the oral mucosal fibroblasts leading to fibrotic bands in the oral mucosa of OSMF patients [6, 7].

Betel nut chewing is a custom in the Indian subcontinent and OSMF has become a significant concern now-a-days due to its malignant transformation. In 2018, Khan confirmed in his study that there is a link between copper and fibrosis in OSMF disease [8]. Furthermore, the quality-of-life hamper greatly in patients with oral submucous fibrosis, particularly when patient's mouth opening gets progressively restricted. Treatment of OSMF is difficult due to its irreversibility. Surgery is mandatory in advanced case of trismus due to its morbidity. So, prevention is important as no healing can be achieved with available treatment.

The purpose of this study was to find out the relation of serum Copper level with oral submucous fibrosis patients. Measurement of copper level in the serum of patients with OSMF may help in early detection of the disease and staging of OSMF status. Thus, it helps to provide proper management and monitoring of the efficacy of treatment. Also, it helps to counseling the patient to discontinue their habit.

## Materials and Methods

It was a case control study conducted from March 2020 to February 2021 in the Department of Oral and Maxillofacial Surgery, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Sample was taken by consecutive sampling and patients with signs and symptoms of OSMF and definitive habit of betel nut chewing were included in the study. Patients suffering from any systemic diseases like renal diseases, liver diseases, cardiac diseases, hematological disorders and other malignancies and taking antioxidants/multivitamin preparations were excluded. The control group were relatively healthy and no history of betel quid chewing and co-morbidity.

Detailed histories of all patients were taken and clinical examination was done by the investigator. Case group diagnosed as OSMF based on clinical presentation such as burning sensation in the mouth, blanching of buccal, retromolar and palatal mucosa, palpable fibrotic band and progressive inability to open the mouth. A clinical staging procedure was applied for OSMF where in stage I, maximum interincisal mouth opening (MIMO) is >35 mm, in stage II, it is 25-35 mm, stage III means 15-25 mm, stage IV is 5-15 mm and stage V is <5mm [9].

In this study 10 patients were taken as case having signs of OSMF, those having definitive history of exclusively betel nut chewing and control group has 18 healthy individuals. Sample collection procedure was explained to the subjects, and written consent was signed by them. For sample collection, 5 ml venous bloods were collected from both groups and serum was separated from blood with centrifuge for 15 minutes at 2500 rpm. Then serum was transferred to Atomic Energy Centre Laboratory.

Serum Cu level was estimated by Flame atomic absorption spectrophotometer (Varian AA240FS, USA) equipped with an air-acetylene flame was used for absorbance measurements at a wavelength of 324.8 nm. The instrumental parameters were adjusted according to the manufacturer's recommendations. By this, the sample was aspirated in through a small tube and transported to the nebulizer where it was broken up into a fine aerosol. Then, the aerosol was carried to the flame, by a carrier gas and broken up into its individual atoms. Then the value was counted by computer-based software.

Serum Cu level data of both groups that is received from Atomic Energy Centre Laboratory report and clinical data (age, gender and stage of OSMF) from history sheet were sorted, tabulated and statistically analyzed. Normal range of serum copper level was considered as 70–140 µg/dL (male) and 80–150 µg/dL (female) [10]. The mean serum copper level of both groups and their standard deviation was calculated by relevant statistical formula. Mean copper levels in different stage of OSMF patients were also calculated.

Statistical analysis was performed by Computer based Software SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL) using independent unpaired Sample *t*-test for quantitative variables (the mean copper level based on the staging of OSMF). A *p*-value of less than 0.05 was considered statistically significant.

Ethical clearance for the study was taken from institutional review board (IRB), BSMMU, Dhaka, Bangladesh prior to the commencement of the study. After the research protocol was approved by the committee, permission for the study was taken from the Department of Oral & Maxillofacial Surgery, BSMMU, Dhaka.

## Results

The minimum and maximum age of OSF patient at presentation was 21 years and 65 years respectively. Mean value is $\bar{x}$ =39.10 and standard deviation is σ =14.41. The majority of patients were found in the group who had in third and fifth decade of their life. (Table 1)

Among the 10 OSF patients, 03 were male and 07 were female patients, thus showing female predominance over male with the ratio of 2.33:1. Female predominance can be related to easy accessibility and socially acceptable for females to use betel nut more frequently than males in our society (Table 2).

An increase in serum copper level there was gradual increase in severity of clinical features and mouth opening progressively decreased. (Table 3)

There is a gradual increase in serum copper level as the clinical stage of OSF increased and subsequent mouth opening gradually decreased (Table 4).

The serum copper level was significantly ($p< 0.001$) elevated among the cases (*126 ±16.06*) than controls (*91.38 ±6.92*) (Table 5).

**Table 1:** Distribution of patients by their Age

| Age (years) | Number of patients (n) | |
|---|---|---|
| | Case group | |
| | n | % |
| 20-29 | 3 | 40.00 |
| 30-39 | 2 | 20.00 |
| 40-49 | 3 | 20.00 |
| 50-59 | 1 | 10.00 |
| 60-69 | 1 | 10.00 |
| Total (N) | 10 | 100 |
| Mean ± SD (Min – Max) | (39.10±14.41) 21 – 65 | |

**Table 2:** Distribution of patients by their gender

| Groups (years) | Gender distribution | | | |
|---|---|---|---|---|
| | Case | | Control | |
| | n | % | n | % |
| Female | 07 | 70.00 | 10 | 55.55 |
| Male | 03 | 30.00 | 08 | 44.44 |
| Total (N) | 10 | 100 | 18 | 100 |

**Table 3:** Clinical feature with serum copper concentrations (µg/dl) in OSF group

| Case Group (Age, Sex) | Duration/ History of Betel Nut chewing (years) | Maximum Mouth opening (mm) | Clinical feature | | | | Clinical stage | Cu level (µg/dl) |
|---|---|---|---|---|---|---|---|---|
| | | | Burning sensation | Blanching of oral mucosa | Fibrotic band | Trismus | | |
| **Case-1 (45, M)** | 6 | 30 | | √ | √ | | 2 | 127 |
| **Case-2 (60, F)** | 3.5 | 33 | | √ | √ | | 2 | 123 |
| **Case-3 (28, F)** | 6 | 33 | | √ | √ | | 2 | 124 |
| **Case-4 (40,F)** | 10 | 15 | | √ | √ | √ | 3 | 152 |
| **Case-5 (41,F)** | 2 | 25 | | √ | √ | √ | 3 | 139 |
| **Case-6 (65,F)** | 10 | 28 | | √ | √ | | 2 | 135 |
| **Case-7 (26,M)** | 2 | 37 | √ | | | | 1 | 105 |
| **Case-8 (35,F)** | 3 | 40 | √ | | | | 1 | 113 |
| **Case-9 (30,M)** | 2 | 39 | √ | | | | 1 | 102 |
| **Case-10 (21,F)** | 3 | 17 | | √ | √ | √ | 3 | 140 |

**Table 4:** Mean copper level in different stage of OSF

| Clinical Stage: | Number of Patients (n) | Mean copper level(**µg/dl**) ±SD |
|---|---|---|
| Stage I | 3 | 106.66±3.50 |
| Stage II | 4 | 127.25 ±2.45 |
| Stage III | 3 | 143.66 ±7.23 |

**Table 5:** Descriptive Statistics of mean serum copper concentrations (µg/dl) in OSF and control group

| Element | Study Group | Number of patients (n) | Mean±SD (µg/dl) | 95%CI | *t* value | *p** |
|---|---|---|---|---|---|---|
| Serum Copper | Case | 10 | 126 ±16.06 | 23.96-45.28 | 6.49 | < 0.001 |
| | Control | 18 | 91.38±6.92 | | | |

***The result is significant at $p < 0.05$

## Discussion

In the present study, 10 subjects with OSMF were in the age range of 21–65 years with a mean age of 39.10 (SD=±14.41) years (Table 1). In 2015, Yadav *et al*. showed that more than half of the cases (58%) and controls (84%) were below 30 years [11]. The age range seen in this study was greater than that of their study. This can be attributed to get more freedom later in life.

Among the 10 OSMF subjects, 03 were male and 07 were female patients, thus showing female predominance over male with the ratio of 2.33:1 (Table 2). According to Khan there is a marked male predominance in OSMF, that he had observed in his study (70% males and 30% females) [8]. It has been found not consistent with present study and concludes increased consumption of betel nut among females as compared to males. Female predominance can be related to easy accessibility and socially acceptable for females to use betel nut more frequently than other deleterious habits.

Furthermore, there is a gradual increase in serum copper level as the clinical stage of OSMF increased (Table 4). It is also consistent with other similar studies. Kumar showed in his study that, the mean serum copper level was lowest for Stage I (116.2 ± 9.9), followed by Stage II (132.9 ± 13.4) and was highest for Stage III (152.3 ± 14.0) [3]. Thus, it could be concluded that, there is a direct relation between serum copper and severity of fibrosis. The results of present study showed that the serum copper level was significantly ($p< 0.001$) higher among the cases (126 ±16.06) than controls (91.38 ±6.92) (Table 5). Several studies in the past have also shown similar results. The serum copper level was significantly ($p< 0.0001$) higher among cases (155.50 ± 40.13) than controls (100.40 ± 24.52) [11].

The study has many limitations. It has small sample size due to COVID-19 pandemic situations. Most patients came from remote area to seek their better and reliable treatment. As the OSMF is not an emergency condition, it seems that patient did not able to come to seek their treatment by overcoming the restrictions of lockdown due to COVID-19 pandemic situations. Moreover, it is a single centered study and the study was dependent on clinical presentation and clinical staging. Although several researchers in the past also performed their study based on clinical staging [3, 12].

## Conclusion

There is an increase in serum copper level in study group compared to control group and there is also an increase in copper level with the advancement of clinical staging of OSMF. From this study it can be concluded that there is an association of serum Copper level with the development of OSMF in patients having betel nut chewing habit. Further research with larger sample size and multi-centered study might be helpful for us to know better about the relation of serum copper level and OSMF.

## Ethical Issue

This study was performed after taking ethical clearance from Institutional Review Board of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

## Conflict of Interest

Authors declare no conflict of interest.

**References**


1. Thakur M, Guttikonda VR. Estimation of hemoglobin, serum iron, total iron-binding capacity and serum ferritin levels in oral submucous fibrosis: A clinicopathological study. Journal of Oral and Maxillofacial Pathology. 2017; 21:30-35. Doi: 10.4103/jomfp.JOMFP_131_15
2. Rao NR, Villa A, More CB, Jayasinghe RD, Kerr AR, Johnson NW. Oral submucous fibrosis: A contemporary narrative review with a proposed inter-professional approach for an early diagnosis and clinical management. J Otolaryngol Head Neck Surg. 2020; 49(1):p3. Doi: 10.1186/s40463-020-0399-7
3. Kumar H, Kumar P, Jain S, Suryawanshi H. Analysis of serum copper and iron levels in oral submucous fibrosis patients: A case-control study. Indian Journal of Dental Sciences. 2016; 8:145-149. Doi: 10.4103/0976-4003.191728
4. Trivedy CR, Warnakulasuriya KA, Peters TJ, Senkus R, Hazarey VK, Johnson NW. Raised tissue copper levels in oral submucous fibrosis. Journal of oral pathology & medicine: Official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2000; 29:241-248. Doi: 10.1034/j.1600-0714.2000.290601.x
5. Guruprasad R, Nair PP, Singh M, Singh M, Singh M, Jain A. Serum vitamin c and iron levels in oral submucous fibrosis. Indian Journal of Dentistry. 2014; 5:81-85. Doi: 10.4103/0975-962X.135266
6. Sachdev PK, Freeland-Graves J, Beretvas SN, Sanjeevi N. Zinc, Copper, and Iron in Oral Submucous Fibrosis: A Meta-Analysis. International Journal of Dentistry, 2018. Doi: 10.1155/2018/3472087
7. 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; 6:e569-e575. Doi:10.4317/jced.51318
8. Khan H. Evaluation of Serum Copper (Cu), Serum Iron (Fe) and Serum Copper (Cu) / Iron (Fe) Ratio in Oral Submucous Fibrosis in Karachi. Journal of Advances in Medicine and Medical Research. 2018; 26:1-9. Doi: 10.9734/JAMMR/2018/40845
9. Passi D, Bhanot P, Kacker D, Chahal D, Atri M, Panwar Y. Oral submucous fibrosis: Newer proposed classification with critical updates in pathogenesis and management strategies. National Journal of Maxillofacial Surgery. 2017; 8:89-94. Doi:10.4103/njms.NJMS_32_17
10. Siddiqui I, Farooqi JQ, Shariff DA. Serum copper levels in various diseases: A local experience at Aga Khan University Hospital, Karachi. International Journal of Pathology. 2006; 4:101-104. https://www.yumpu.com/user/jpathology.com
11. Yadav A, Kumar L, Misra N, Deepak U, Shiv Kumar GC. Estimation of serum zinc, copper, and iron in the patients of oral submucous fibrosis. National Journal of Maxillofacial Surgery. 2015; 6:190-193. Doi: 10.4103/0975-5950.183851
12. Arakeri G, Hunasgi S, Colbert S, Merkx MA, Brennan PA. Role of drinking water copper in pathogenesis of oral submucous fibrosis: A prospective case control study. The British Journal of Oral & Maxillofacial surgery. 2014; 52:507-512. Doi: 10.1016/j.bjoms.2014.03.023