

EVALUATION OF SERUM COPPER, ZINC, IRON AND MAGNESIUM LEVELS IN PATIENTS WITH ORAL SUBMUCOUS FIBROSIS: A HOSPITAL-BASED COMPARATIVE CASE-CONTROL STUDY

Dr. Manish Kumar Jain¹, Dr. Abhishek Singhvi², Dr. Gurdeep Singh³, Dr. Neeraj Saraswat⁴, Dr. Mahesh Meshram⁵

¹Post Graduate student, Vyas Dental College & Hospital, Jodhpur, dr.manishagra@gmail.com, Rajasthan University of Health Sciences

²Professor & Head of Department, Vyas Dental College & Hospital, Jodhpur, Rajasthan University of Health Sciences, Jaipur

³Professor, Vyas Dental College & Hospital, Jodhpur, Rajasthan University of Health Sciences, Jaipur

⁴Post Graduate student, Vyas Dental College & Hospital, Jodhpur, Email-drsaraswat@gmail.com, Rajasthan University of Health Sciences, Jaipur

⁵Post Graduate student, Vyas Dental College & Hospital, Jodhpur, Rajasthan University of Health Sciences, Jodhpur

ABSTRACT

Background: Oral Submucous Fibrosis (OSMF) is a long-lasting and progressive disorder that can become cancerous. It is mainly linked to chewing areca nut and smokeless tobacco. The condition causes the tissues in the mouth to become stiff, which leads to limited mouth opening, a burning feeling, and a higher risk of cancer. Changes in certain trace elements in the blood have been connected to how OSMF develops and could help track the disease or catch it early.

Aim: To estimate and compare the serum levels of trace elements—copper, zinc, iron, and magnesium—in patients with Oral Submucous Fibrosis and healthy individuals.

Materials and Methods: A hospital-based comparative case-control study was conducted in the Department of Oral Pathology, Vyas Dental College and Hospital, Jodhpur. A total of 50 participants were enrolled, comprising 25 clinically diagnosed OSMF patients and 25 age- and sex-matched healthy controls. The clinical diagnosis was based on a detailed history and characteristic oral manifestations. Venous blood samples were collected, serum was separated by centrifugation, and trace element concentrations (copper, zinc, iron, and magnesium) were estimated using standard reagent kits with a semi-automatic analyzer. Statistical analysis was performed using SPSS Version 15.0. Independent t-test, Chi-square test, and one-way ANOVA were applied, with a p-value ≤ 0.05 considered statistically significant.

Results: OSMF predominantly affected young adults, with the majority of patients belonging to the 21–40-year age group, and demonstrated a marked male predominance. Serum copper levels were significantly higher in OSMF patients compared with healthy controls. In contrast, serum zinc, iron, and magnesium levels were significantly lower in the OSMF group ($p \leq 0.05$). Serum copper showed a significant positive association with the duration of chewing habits, while serum zinc also demonstrated a significant association. Iron and magnesium did not show a statistically significant correlation with habit duration.

Conclusion: The present study demonstrates a significant imbalance in serum trace element levels among patients with Oral Submucous Fibrosis. Elevated serum copper and decreased serum zinc, iron, and magnesium levels may contribute to the pathogenesis and progression of OSMF. These findings suggest that serum trace element estimation may serve as a simple, non-invasive, and cost-effective adjunctive biomarker for the early diagnosis and monitoring of OSMF. Further studies with larger sample sizes are recommended to validate these observations.

KEYWORDS: Oral Submucous Fibrosis, OSMF, Trace Elements, Copper, Zinc, Iron, Magnesium, Areca Nut, Biomarkers, Oral Potentially Malignant Disorder.

INTRODUCTION

Abstract: Oral Submucous Fibrosis (OSMF) is an insidious, chronic, progressive disease of the oral cavity which is not truly inflammatory in nature and with juxta-epithelial inflammatory changes leading to a resultant stiffness of the oral mucosa and eventually limited mouth opening. The disease is mostly limited to the buccal mucosa but may also be present in the soft palate, lips, pharynx and upper oesophagus. Burning sensation intolerance to spicy food reduced mouth opening mastication dysphagia and speech related issues are frequently encountered by the patients making them miserable in quality of life. The WHO has classified OSMF as an Oral Potentially Malignant Disorder (OPMD) owing to the evidence of severe risk of malignant transformation into oral squamous cell carcinoma. [1–3]

Schwartz (1952) was the first to describe the condition, followed by Pindborg and Sirsat giving the classical histopathological definition of Oral Submucous Fibrosis in 1966. Since then, OSMF has evolved into a major public health epidemic in countries like South and Southeast Asia where chewing areca nut is considered part of a lifestyle or culture. Areca nut is the fourth most common psycho active substance in the world and since India contributes to almost half of the disease burden among the 3,333 million areca nut consumers globally due to its commercialized form (gutkha, pam masala, betel quid) [1]. [1,2]

Areca nut is recognized as the main cause of OSMF with abundant epidemiological studies. Areca nut constitutes several alkaloids of which arecoline stimulates fibroblast proliferation, promotes collagen overproduction with breakdown activity suppressed leading to advancing oral mucosal fibrosis. OSMF risk is considerably greater in more regular and prolonged areca nut chewing, and commercialised processed preparations of areca nut have an estimated 10-fold higher concentration per preparation than areca nut directly from the market, rendering these products much more dangerous than traditional betel quid. [2,4]

While areca nut is the principal etiological agent, OSMF is of multifactorial origin. Deficient nutrition, genetic predisposition, and immunopathological abnormalities have been linked to disease advancement along with increased oxidative stress, modified collagen metabolism, and inflammatory responses. Recently, advances in molecular biology have shown that dysregulated cytokines, transforming growth factor-beta (TGF- β), matrix metalloproteinases, and tissue inhibitors of metalloproteinases play a major role in the pathogenesis of excessive deposition of extracellular matrix (ECM) leading to the development and progression of fibrosis. [4,5]

Trace elements are micronutrients that play key regulatory roles in many biochemical and enzymatic reactions involved in cell metabolism, connective tissue production, antioxidant defense system activity, and immune regulation. Normal tissue homeostasis is regulated by metals such as copper, zinc,[2] iron[3] and magnesium. These are trace elements identified as possible markers of disease activity and progression following variations in serum concentrations reported in many chronic inflammatory disorders and malignancies. In the case of copper, it acts as a cofactor for lysyl oxidase—an enzyme important in collagen cross-linking; whereas zinc and iron are part of epithelial repair, antioxidant mechanism, and immune functions. Magnesium is essential for cellular metabolism and normal tissue physiology. [6,7]

Elevated serum concentrations of copper with decreased concentrations of zinc and iron have been reported by several investigators in OSMF patients. Higher levels of copper are linked to the high copper content of betel nut, which stimulates collagen production and encourages fibrosis. In contrast, lower serum zinc and iron levels can be attributed to increased consumption in collagen synthesis, dietary deficiencies or as a consequence of chronic inflammatory changes. Such biochemical changes may represent disease severity and are likely to be useful markers for tracking disease evolution and the risk of malignant change. [6–8]

More recent studies have highlighted the role of serum trace elements, in adding value to the diagnosis and prognosis of OSMF. Changes in some trace elements like copper, zinc, and iron as well as other micronutrients have been linked to the development of fibrosis and malignant transformation, making them potential non-invasive biomarkers. The assessment of serum trace elements may assist in early diagnosis of progression to the disease, and help with patient management due to convenience, inexpensive cost and low invasiveness properties of blood based investigations. [8–10]

In light of the rising incidence of 'Oral Submucous Fibrosis' (OSMF) in young adults and a lack of satisfactory positive biochemical markers of disease progression, we undertook this study to evaluate serum levels of Copper Zinc, Iron and Magnesium among patients with Oral Submucous Fibrosis as well as healthy controls. These findings may shed further light on the role of trace elements in the pathogenesis of OSMF as well as possibly provide supportive biomarkers in clinical practice. [3,5,10]

METHODOLOGY

The present hospital-based comparative case-control study was conducted in the Department of Oral Pathology, Vyas Dental College and Hospital, Jodhpur, Rajasthan, after obtaining approval from the Institutional Ethics Committee. A total of 50 participants were enrolled, including 25 clinically diagnosed OSMF patients (Group I) and 25 age- and sex-matched healthy controls (Group II). Written informed consent was obtained from all participants before enrolment.

The diagnosis of OSMF was based on detailed clinical history and characteristic oral manifestations, including burning sensation, blanching of the oral mucosa, palpable fibrous bands, and restricted mouth opening. Approximately 5 mL of venous blood was collected aseptically from each participant. The blood samples were centrifuged to separate the serum, and the serum levels of copper, zinc, iron, and magnesium were estimated using standard reagent kits with a semi-automatic analyzer.

The collected data were analyzed using SPSS Version 15.0. Descriptive statistics were expressed as mean and standard deviation. The Independent Student's t-test, Chi-square test, and one-way ANOVA were used for statistical analysis. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

A total of 50 participants were enrolled in the present hospital-based comparative case-control study, comprising 25 clinically diagnosed Oral Submucous Fibrosis (OSMF) patients and 25 age- and sex-matched healthy controls. The demographic profile, clinical characteristics, and serum trace element levels were evaluated and compared between the two groups (Table 1).

The majority of OSMF patients belonged to the 21–40 years age group, indicating that the disease predominantly affected young adults. A marked male predominance was observed among OSMF patients, with males accounting for the majority of the study population (Tables 2 and 3).

Biochemical analysis demonstrated a significant increase in mean serum copper levels among OSMF patients compared with healthy controls. In contrast, the mean serum zinc, iron, and magnesium levels were significantly reduced in the OSMF group. These differences were found to be statistically significant ($p \leq 0.05$) (Table 4).

Further evaluation of the relationship between trace element levels and the duration of chewing habits revealed that serum copper levels increased significantly with increasing duration of the habit. Serum zinc levels also demonstrated a

statistically significant association with habit duration. However, serum iron and magnesium levels did not show any statistically significant correlation with the duration of chewing habits ($p > 0.05$) (Table 5). Overall, the findings indicate that alterations in serum trace element levels, particularly elevated serum copper and reduced serum zinc, iron, and magnesium levels, are significantly associated with Oral Submucous Fibrosis and may serve as useful biochemical markers for disease assessment and progression (Tables 4 and 5).

Results Tables (Sample Size = 50)

Table 1. Distribution of Study Participants

Group	Number (n)	Percentage (%)
OSMF Patients	25	50
Healthy Controls	25	50
Total	50	100

Table 2. Age-wise Distribution of OSMF Patients (n = 25)

Age Group (Years)	Number	Percentage (%)
21–30	9	36
31–40	8	32
41–50	4	16
51–60	2	8
61–70	2	8
Total	25	100

Table 3. Gender-wise Distribution of OSMF Patients (n = 25)

Gender	Number	Percentage (%)
Male	19	76
Female	6	24
Total	25	100

Table 4. Comparison of Mean Serum Trace Element Levels Between OSMF Patients and Healthy Controls

Trace Element	OSMF Patients (Mean $\pm$ SD)	Healthy Controls (Mean $\pm$ SD)	P-value
Serum Copper ($\mu\text{g/dL}$)	184.22 $\pm$ 15.47	101.04 $\pm$ 11.25	0.05*
Serum Zinc ($\mu\text{g/dL}$)	55.45 $\pm$ 12.16	98.13 $\pm$ 9.21	0.02*
Serum Iron ($\mu\text{g/dL}$)	56.24 $\pm$ 14.22	102.37 $\pm$ 13.18	0.001*
Serum Magnesium (mEq/L)	0.88 $\pm$ 0.12	1.71 $\pm$ 0.36	0.03*

Statistically significant at $p \leq 0.05$.

Table 5. Correlation of Serum Trace Elements with Duration of Chewing Habits in OSMF Patients

Trace Element	1–10 Years	11–20 Years	>20 Years	P-value
Serum Copper ($\mu\text{g/dL}$)	179.21 $\pm$ 1.40	183.14 $\pm$ 10.05	195.14 $\pm$ 4.21	0.001*
Serum Zinc ($\mu\text{g/dL}$)	48.20 $\pm$ 3.55	54.47 $\pm$ 10.20	64.32 $\pm$ 5.01	0.05*
Serum Iron ($\mu\text{g/dL}$)	54.22 $\pm$ 10.05	60.14 $\pm$ 9.10	67.23 $\pm$ 11.02	0.25
Serum Magnesium (mEq/L)	0.81 $\pm$ 0.20	0.89 $\pm$ 0.14	0.99 $\pm$ 0.80	0.50

Statistically significant at $p \leq 0.05$.

DISCUSSION

Oral submucous fibrosis (OSMF) is a chronic, progressive disorder that is firmly linked to areca nut chewing and has variable potential for malignant transformation. This study is a cross-sectional case-control which has evaluated the serum levels of copper, zinc, iron and magnesium between OSMF patients and healthy controls. Results indicated that serum copper levels were significantly elevated and serum zinc, iron and magnesium levels were significantly decreased in patients with OSMF as compared to control subjects suggesting that an underlying disturbance of trace element metabolism may be important in the pathogenesis and progression of this disease. [11,12]

In the present study, majority of OSMF patients belonged to the age group of 21–40 years & showed a definite male preponderance. Such observations have been previously reported by Chhabra et al. [16] by stating that the OSMF is now on the rise among younger people as more are consuming commercially available forms of betel quid products like gutkha

and pan masala. Epidemiological studies have recently also found a greater prevalence of the disease among males due to increased exposure in chewing habits. [17,18]

Plasma copper level was elevated in the current study. Copper is a cofactor for lysyl oxidase, which catalyzes collagen cross-linking and thus leads to increased deposition of collagen and fibrosis. Sachdev et al. have also observed similar results [11], Arakeri et al. [12], and Mekala et al. Wangsadip et al.

However, serum zinc level was significantly reduced in OSMF patients. Zinc is an essential mineral that it plays a significant role in epithelial repair, antioxidant defense, immune regulation and preservation of normal cellular metabolism. Decreased zinc levels to damage the tissue and enhance oxidative stress that make it possible for fibrosis development. Such observations have been also noted in the systematic review by Sachdev et al. [11] and the recent Mekala et al. There were two comparative studies from India [17] showing relatively low serum zinc level in the patients with OSMF as compared to the healthy controls.

In the current study OSMF patients also demonstrated significantly reduced serum iron levels. Iron deficiency, although often reported in patients with OSMF, could be attributed to poor dietary intakes caused by an inability to take solid food due to restricted mouth opening, chronic inflammation and increased utilization of iron during collagen synthesis. These results do correlate well with the previous observations made by Chhabra et al. [16] and Saoji et al. [18] which postulated iron deficiency could lead to epithelial atrophy thereby predisposing the oral mucosa to fibrosis and malignant transformation.

Likewise, serum magnesium values were also much less in OSMF individuals than in healthy controls. Magnesium is important for many enzyme reactions, cellular metabolism and its role in tissue integrity. While only few studies have explored magnesium in OSMF, emerging evidence shows that disturbed magnesium metabolism may be involved into oxidative stress and chronic inflammatory changes seen due to the disease. These observations corroborated the recent reports exploring the contributions of trace elements in OSMF. [17,18]

Our results also revealed that serum levels of copper and zinc were significantly correlated with the brachycephalic duration of chewing habits, while there was no statistically significant association between serum iron and magnesium. Conclusion: These results suggest that chronic exposure to areca nut may lead to gradual changes in copper and zinc metabolism, resulting in enhanced collagen production and fibrosis. Mekala et al. have reported similar findings Design of the study: Survey, Contribution: by Hernández-Guerrero et al., [16] they found imbalances in trace elements associated to chewing habits become more pronounced when prolonged over time [17].

Materials and methodsRecent literature suggests trace elements as promising biomarkers for early disease diagnosis and monitoring. Before including the review by Pradnyesh et al. in this scoping exercise, The increasing interest in salivary and serum biomarkers for OSMF was highlighted by [19], while Chhabra et al. Biochemical markers may aid in the early diagnosis and timely intervention [16]. Furthermore, Tilakaratne et al. This imbalance of trace elements along with oxidative stress, persistent inflammation and aberrant collagen metabolism and the interplay between these processes play an important role in the pathogenesis of OSMF and its malignant transformation according to [20].

Hence, the results of the current study tally with an existing literature and further substantiate serum trace element estimation as a simple, non-invasive and cost-effective adjunctive investigation in patients with Oral Submucous Fibrosis. Nevertheless, the limited sample size and single-center study design may limit the generalizability of these findings. Large multicentric studies with adequate sample size and longer follow up is required to confirm the clinical utility of serum trace elements as prognostic markers for disease progression and malignant transformation in OSMF. [16–20]

CONCLUSION

This study showed that Oral Submucous Fibrosis patients had a significantly higher serum copper level and lower serum zinc, iron, and magnesium levels than those of healthy control participants. Elevated serum copper and decreased serum zinc were highly associated with the duration of chewing habits, which may correlate with their role in the pathogenesis and progression of OSMF. Measurement of serum trace elements could be a straightforward, low-cost and non-invasive complementary biomarker for diagnosis and monitoring of disease progression. But, larger study investigations recommended those findings must be established.

REFERENCES

1. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. *Oral Surg Oral Med Oral Pathol.* 1966;22(6):764-779. doi: 10.1016/0030-4220(66)90367-7.
2. Pindborg JJ, Mehta FS, Gupta PC, Daftary DK. Prevalence of oral submucous fibrosis among 50,915 Indian villagers. *Br J Cancer.* 1968;22(4):646-654. doi: 10.1038/bjc.1968.76.
3. Ranganathan K, Devi MU, Joshua E, Kirankumar K, Saraswathi TR. Oral submucous fibrosis: a case-control study in Chennai, South India. *J Oral Pathol Med.* 2004;33(5):274-277. doi: 10.1111/j.0904-2512.2004.00116.x.
4. Rajendran R. Oral submucous fibrosis: etiology, pathogenesis, and future research. *Bull World Health Organ.* 1994;72(6):985-996. PMID: 7857821.
5. Tadakamadla J, Kumar S, Mamatha GP. Evaluation of serum copper and iron levels among oral submucous fibrosis patients. *Med Oral Patol Oral Cir Bucal.* 2011;16(7):e870-e873. doi: 10.4317/medoral.17083.
6. Khanna SS, Udas AC, Kumar GK, Suvarna S, Karjodkar FR. Trace elements (copper, zinc, selenium and molybdenum) as markers in oral submucous fibrosis and oral squamous cell carcinoma. *J Trace Elem Med Biol.* 2013;27(4):307-311. doi: 10.1016/j.jtemb.2013.04.003.
7. Ankolekar M, Patil S, Rao RS. Estimation of the serum and the salivary trace elements in OSMF patients. *J Clin Diagn Res.* 2013;7(7):1215-1218. doi: 10.7860/JCDR/2013/5207.3023. PMID: 23905144.

8. Neethi H, Patil S, Rao RS. Estimation of serum copper and zinc levels in oral submucous fibrosis: An atomic absorption spectroscopic study. *World J Dent.* 2014;5(1):14-17. doi: 10.5005/jp-journals-10024-1406. PMID: 24685778.
9. Prabhu RV, Prabhu V, Chatra L, Shenai P, Suvarna N, Dandekeri S. Areca nut and its role in oral submucous fibrosis. *J Clin Exp Dent.* 2014;6(5):e569-e575. doi: 10.4317/jced.51318.
10. 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. *Natl J Maxillofac Surg.* 2015;6(2):190-193. doi: 10.4103/0975-5950.183851. PMID: 27390495.
11. Sachdev PK, Freeland-Graves J, Beretvas SN. Zinc, copper, and iron in oral submucous fibrosis: A meta-analysis. *Int J Dent.* 2018;2018:3472087. doi: 10.1155/2018/3472087. PMID: 30046309.
12. Arakeri G, Patil SG, Aljabab AS, Lin KC, Merks MAW, Brennan PA. Oral submucous fibrosis: an update on current theories of pathogenesis. *J Oral Pathol Med.* 2017;46(6):406-412. doi: 10.1111/jop.12581.
13. Warnakulasuriya S. Clinical features and presentation of oral potentially malignant disorders. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2018;125(6):582-590. doi: 10.1016/j.oooo.2018.03.011.
14. Kujan O, Farah CS, Johnson NW. Oral and potentially malignant disorders: a concise review. *J Oral Pathol Med.* 2021;50(3):177-186. doi: 10.1111/jop.13141.
15. Abidullah M, Suresh KV, Reddy GS. Hematological profile in oral submucous fibrosis patients. *J Oral Maxillofac Pathol.* 2022;26(Suppl 1):S58-S63.
16. Chhabra AK, Sune R, Reche A. Oral submucous fibrosis: A review of the current concepts in management. *Cureus.* 2023;15(10):e47259. doi: 10.7759/cureus.47259.
17. Mekala MS, Koothati RK, Mummidi K, et al. Evaluation of the role of trace elements in malignant transformation of oral submucous fibrosis: A study in the population of Krishna District, Andhra Pradesh, India. *Cureus.* 2024;16(7):e65314. doi: 10.7759/cureus.65314. PMID: 39184678.
18. Saoji S, Sharma N, et al. The role and significance of trace elements in oral submucosal fibrosis. *Cureus.* 2024;16:e****. PMID: 39036231. PMCID: PMC11259020.
19. Pradnyesh R, et al. Salivary profile in oral submucous fibrosis: A scoping review. *J Health Allied Sci NU.* 2024. PMID: 39102860.
20. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. *Oral Oncol.* 2006;42(6):561-568. doi: 10.1016/j.oraloncology.2005.10.005.