



## Estimation of Serum Copper and Zinc Levels in Oral Submucous Fibrosis: An Atomic Absorption Spectroscopic Study

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### ABSTRACT

**Background and objectives:** Biochemical derangements of microelements although reported in oral cancer is poorly understood in oral submucous fibrosis (OSMF). Hence, the present study was carried out to estimate and compare the serum copper and zinc levels among different histopathological stages of OSMF with that of healthy controls.

**Materials and methods:** Thirty histopathologically diagnosed cases of OSMF and 30 healthy controls reporting to MS Ramaiah Dental College and Hospital (MSRDCH), Bengaluru, were included in the study. The histopathological staging of OSMF was done as per criteria given by Pindborg and Sirsat (1966). Blood samples were collected and the serum copper and zinc levels were estimated using atomic absorption spectroscopy. The results were statistically analyzed using independent t-test and Kruskal-Wallis test.

**Results:** The mean serum copper levels were increased ( $p < 0.0005$ ) and zinc levels were decreased ( $p < 0.01$ ) in OSMF patients when compared to that of controls. The mean serum copper levels showed an increasing trend from stage I to IV ( $p = 0.004$ ) while zinc levels showed a decreasing trend from stage I to IV ( $p = 0.04$ ) in OSMF patients.

**Conclusion:** The serum copper and zinc levels in OSMF showed a significant difference in comparison to that of controls and between the histopathological stages. These findings indicate that serum copper and zinc may have a contributory role in the etiopathogenesis of OSMF.

**Clinical relevance:** Although the role of the trace elements as etiological factors is minor, derangements noted in the serum copper and zinc levels could be correlated with diseased progression and possibly explain the transformation of OSMF into malignancy. Thus, they can be used as prognostic markers and can be of value for proactive intervention.

**Keywords:** Atomic absorption spectroscopy, Oral submucous fibrosis, Serum copper, Serum zinc.

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**Conflict of interest:** None declared

### INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, progressive scarring disease first described by Schwartz (1952).<sup>1</sup> The association of this condition is predominantly seen in Indians and Southeast Asians<sup>2</sup> with an overall prevalence rate in India of 0.2 to 0.5%.<sup>3</sup> OSMF is also a well recognized potentially malignant disorder with a malignant transformation rate of 7 to 13%.<sup>4</sup> Hence, the only hope for controlling OSMF lies in learning more about its etiopathogenesis.

Micronutrients particularly antioxidant minerals like selenium, copper, zinc, manganese, iron are risk modifiers of cancer.<sup>5</sup> Copper is an essential trace element required for the functioning of several key enzymes like cytochrome-c-oxidase, superoxide dismutase, metallothionein and lysyl oxidase.<sup>6</sup> Zinc is implicated in modulation of mucosal metallothionein interfering with copper absorption.<sup>7</sup> The serum levels of copper and zinc have been reported to be altered in many malignant and nonmalignant conditions<sup>8</sup> but the biochemical derangements in these trace elements and its association with OSMF is poorly understood.

Therefore, the aim of the present study was to estimate and compare the serum copper and zinc levels among different histopathological stages of OSMF with that of healthy controls.

### MATERIALS AND METHODS

A cross-sectional study was carried out on 30 diagnosed cases of OSMF and 30 age and gender-matched controls reporting to the MSRDCH, Bengaluru. Ethical clearance was obtained from the ethical committee, MSRDCH and an informed

written consent was taken from all the subjects. Patients with definitive clinical and histopathological diagnosis of OSMF were included in the study. OSMF patients with associated systemic diseases, malignant transformation, other oral mucosal lesions; history of copper or zinc supplementation in past 1 year and patients undergoing treatment for OSMF were excluded from the study.

The patients with OSMF were staged histopathologically based on the criteria given by Pindborg and Sirsat (1966) (Table 1).<sup>9</sup> A total of 5 ml of venous blood was drawn under aseptic precautions. The blood was allowed to clot for 1 hour and centrifuged at 1,000 rpm for 15 minutes to obtain the serum. The serum samples were preserved in a frozen state (-20°C) until analysis, in a deep freeze refrigerator.

### Estimation of Serum Copper and Zinc Levels by Atomic Absorption Spectroscopy (AAS)

The stored serum samples were brought to room temperature and then diluted with 1% nitric acid (1:5 dilution—1 ml of serum in 4 ml of 1% nitric acid) for deproteinization. Estimation of the serum copper and zinc content was carried out by AAS (Electronic Corporation of India Limited; model 492) (Fig. 1).

AAS procedure is based upon the fact that metal atoms absorb light energy strongly at discrete characteristic wavelength which coincides with the emission spectra line of that particular element (Fig. 2). For estimation of serum copper and zinc, a hollow cathode lamp of copper and zinc were used. The hollow cathode lamp emits a spectrum where the beam traverses the flame and is focused on the entrance slit of a monochromator which is set to read the intensity of a spectral line. Light with this wavelength is absorbed by the flame. For elemental analysis working standards of 0, 0.25, 0.5, 0.75 and 1 µg/ml for copper and zinc were prepared. By measuring the absorbance of the working standards of known concentration of the element, the concentration of the element in the serum sample was estimated at wavelength for copper being 324.8 nm (Fig. 3) and for zinc being 213.9 nm (Fig. 4).<sup>10,11</sup>

Data was entered in Microsoft excel and analyzed using statistical package for social services (SPSS), Chicago. Descriptive data that included mean, standard deviation and percentages were calculated for each group. Comparison



Fig. 1: Atomic absorption spectrophotometer

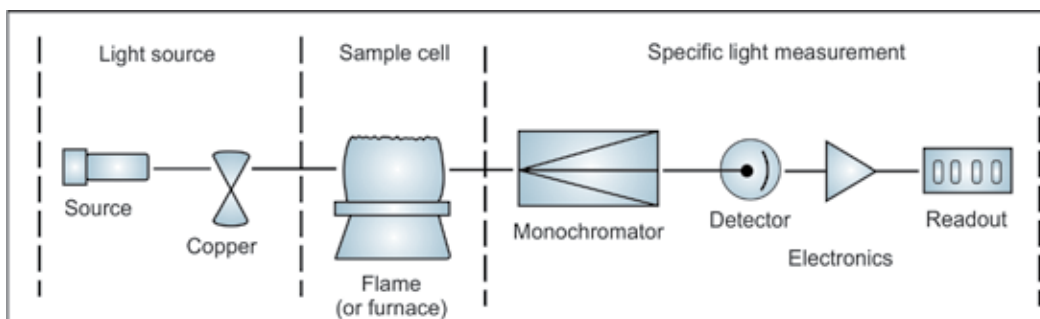


Fig. 2: Schematic diagram showing principle of atomic absorption spectrophotometer<sup>11</sup>

Table 1: Classification based on histopathological features of OSMF<sup>9</sup>

Stages	Histopathological features
Very early stage	Finely fibrillar collagen dispersed with marked edema, plump young fibroblasts with abundant cytoplasm, blood vessels are dilated and congested, inflammatory cells mainly polymorphonuclear leukocytes with occasional eosinophils.
Early stage	Juxta-epithelial area shows early hyalinization. Collagen still in separate thick bundles, moderate number of plump young fibroblasts is present, dilated and congested blood vessels, inflammatory cells are primarily lymphocytes, eosinophils and occasional plasma cells.
Moderately advanced stage	Collagen is moderately hyalinized. Fibroblastic response is less marked, blood vessels are either normal or compressed, inflammatory exudate consists of lymphocytes and plasma cells.
Advanced stage	Collagen is completely hyalinized. Smooth sheets with no separate bundles of collagen. Edema is absent, hyalinized area is devoid of fibroblasts, blood vessels are completely obliterated and narrowed, inflammatory cells are lymphocytes and plasma cells.

between serum copper and zinc levels between OSMF and controls was carried out using independent 't'-test. Multiple group comparisons were made by Kruskal-Wallis test. For

all the tests, a p-value of 0.05 or less was considered as statistically significant.

**RESULTS**

In the present study, the youngest patient was 20 years and the oldest was 70 years with a mean age of  $36.53 \pm 12.12$  years. Out of 30 subjects, eight (27%) were females and 22 (73%) were males. Of 30 subjects, 24 (80%) chewed pan with betel nut, 18 (60%) chewed gutkha, 11 (36.66%) chewed panmasala, eight (26.66%) had the habit of smoking, two (6.66%) had the habit of alcohol.

It was noted that the mean serum copper levels among 30 OSMF subjects was  $126.73 \mu\text{g}/100 \text{ ml} \pm 10.37$  and mean serum zinc levels was  $83.8 \mu\text{g}/100 \text{ ml} \pm 10.90$  when compared to the controls who showed a value of  $120.33 \mu\text{g}/100 \text{ ml} \pm 5.59$  ( $p = 0.004$ ) and  $88.03 \mu\text{g}/100 \text{ ml} \pm 3.11$  ( $p = 0.04$ ) respectively (Table 2 and Graph 1).

The mean serum copper and zinc levels were noted to be different among patients with different histopathological stages. The serum copper in stage I showed the least value ( $104 \mu\text{g}/100 \text{ ml}$ ) while stage IV showed the highest value ( $138 \pm 2.28 \mu\text{g}/100 \text{ ml}$ ) ( $p < 0.0005$ ). On the contrary, the mean serum zinc levels in stage I showed the highest value ( $98 \mu\text{g}/100 \text{ ml}$ ) and stage IV showed the least value ( $71 \pm 9.59 \mu\text{g}/100 \text{ ml}$ ) ( $p < 0.01$ ) (Table 3 and Graph 2).

**DISCUSSION**

The pathogenesis and treatment of OSMF has been a subject of numerous controversies. Biochemical estimation of micronutrients in the sera of patients with precancerous conditions can help not only in determining the pathogenesis but can also be used as indicators of early diagnosis of malignant transformation and prognosis. The

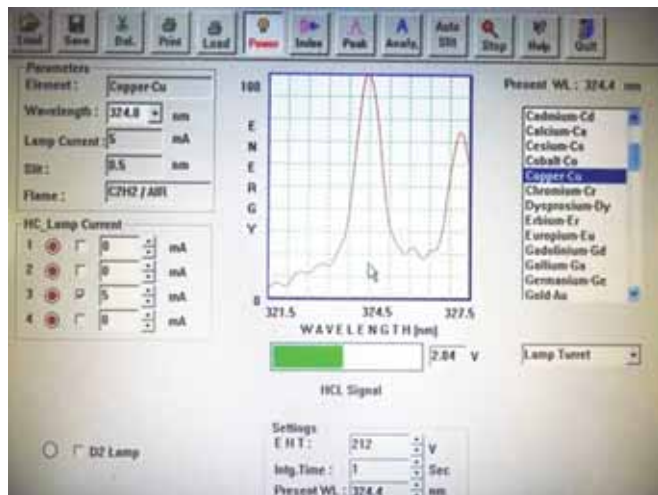


Fig. 3: Wavelength for copper (324.8 nm)

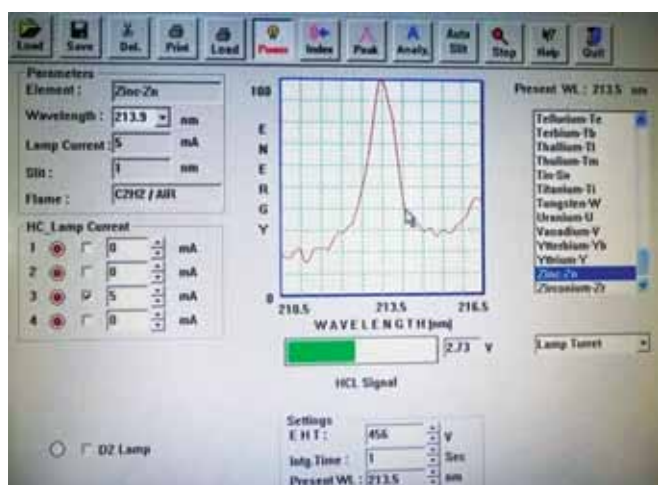


Fig. 4: Wavelength for zinc (213.9 nm)

**Table 2:** Comparison of mean serum copper and zinc levels between OSMF patients and controls

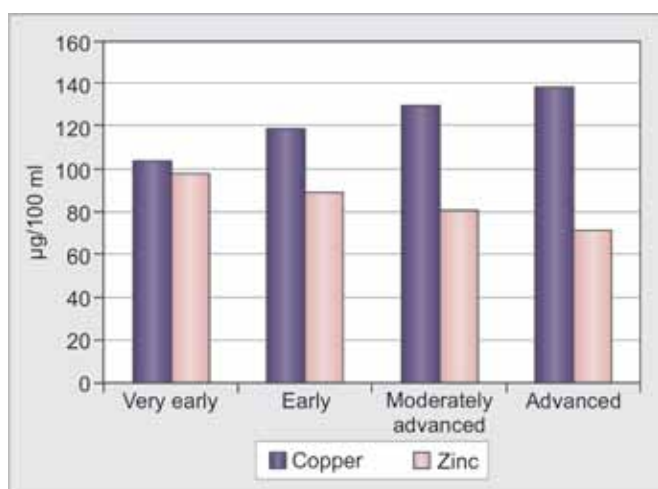
Element	Study group	Mean ( $\mu\text{g}/100 \text{ ml}$ )	Standard deviation	p-value
Copper	OSMF	126.73	10.37	0.004
	Controls	120.33	5.59	
Zinc	OSMF	83.8	10.90	0.04
	Controls	88.03	3.11	

**Table 3:** Comparison of mean serum copper and zinc levels in the different histopathological stages of OSMF patients<sup>9</sup>

Element	Stage	N	Mean ( $\mu\text{g}/100 \text{ ml}$ )	Standard deviation	H	p-value
Copper	Very early	1	104	-	20.08	<0.0005
	Early	9	119.11	10.97		
	Moderately advanced	18	130.55	10.37		
	Advanced	2	138	2.28		
Zinc	Very early	1	98	-	12.26	<0.01
	Early	9	89.77	11.79		
	Moderately advanced	18	81.44	10.90		
	Advanced	2	71	9.59		



**Graph 1:** Mean serum copper and zinc levels between OSMF patients and controls



**Graph 2:** Mean serum copper and zinc levels in the histopathological stages of OSMF patients

technique of estimation of the micronutrients is accurate, reliable and reproducible.

In the present study, the mean serum copper level among 30 OSMF patients was found to be higher compared to the controls. This is in accordance with results obtained by Gupta et al (1987),<sup>8</sup> Shettar and Mubeen (2010),<sup>5</sup> Tadakamadla et al (2011)<sup>12</sup> but in contrast to the normal values obtained in OSMF patients by Trivedy et al (2000)<sup>6</sup> and Ajay Nayak et al (2011).<sup>13</sup> The 30 patients were divided into four histopathological stages and it was noted that the mean serum copper levels increased as the disease progressed. This is in confluent with the study done by Shettar et al (2010) who used Khanna and Andrade classification (1995) where the mean serum copper levels showed an increasing trend through grade I to IV in OSMF patients.<sup>5</sup>

It was noticed that most of the subjects in the present study had the history of using pan with areca nut and it is reported that high levels of copper in areca nut plays an initiating role in stimulation of fibrogenesis.<sup>12</sup> Studies done

by Ma et al (1995), Trivedy et al (2000) and Khanna et al (2006) suggested that increased copper content in areca nut is implicated in tissue fibrogenesis via the copper-dependent enzyme lysyl oxidase, which has a crucial role in cross-linking of collagen and elastin, thereby causing inhibition of degradation of collagen in OSMF patients. Copper is an essential cofactor required for the expression of lysyl oxidase.<sup>6,14,15</sup> The increase in copper may also be attributed to the increased production of copper containing ceruloplasmin by liver as an anti-inflammatory response or due to decrease in catabolism of serum ceruloplasmin.<sup>16</sup>

In our study, the mean serum zinc level among 30 OSMF patients was found to be lower than the controls. This is in accordance with results obtained by Shettar et al (2010).<sup>5</sup> In contrast, studies by Gupta et al (1987),<sup>8</sup> Ajay Nayak et al (2011)<sup>13</sup> showed no significant alteration in the serum zinc levels in OSMF patients. Our study also demonstrated different mean serum zinc levels with different histopathological stages with stage I showing the highest and stage IV showing the least value. This is in contrast to the study by Shettar et al (2010) who used Khanna and Andrade classification (1995) and showed no significant difference in the mean serum zinc levels among the clinical and histological grades in OSMF patients.<sup>5</sup>

Rajendran (2002) noted that the role of copper could not be segregated from that of zinc; and that the biochemical relatedness of these two elements was well elucidated. They also stated that zinc bears an inverse relationship with copper and has been implicated in the modulation of mucosal metallothionein, thereby interfering with the absorption of copper.<sup>7,13</sup>

The present study showed an increasing trend of serum copper and a decreasing trend of serum zinc levels as the stage increased. These variations in the serum copper and zinc levels can possibly be used as prognostic markers for transformation of OSMF into malignancy. The progression of OSMF to cancer may be linked to the mutagenic effects of copper via binding to DNA resulting in p53 aberrations in oral keratinocytes.<sup>6</sup> The decreased serum zinc levels as the stage increases determines the progression of the disease.<sup>5</sup>

As suggested by Tadakamadla, the present study cannot be generalized to all OSMF patients due to regional and cultural variations that occur within and between the countries.<sup>12</sup> The dilemma in the literature regarding these trace elements and its association with OSMF can be minimized further by using a larger sample size. The other limitation could be instrument being expensive and not affordable in all clinical setups. However, on the plus side, the accuracy of atomic absorption spectrophotometer in analyzing the trace elements is exceptional.

## CONCLUSION

It can be concluded from the present study that copper and zinc play a role in the etiopathogenesis of OSMF and can also be used as a prognostic marker. The estimation of serum levels of these trace elements in OSMF patients with a regular follow-up may help in the management of these conditions in an optimized way.

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