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Original Article

Mucosal copper and zinc levels in oral submucous fibrosis patients

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ABSTRACT:

Background: Oral submucous fibrosis (OSMF) is a chronic debilitating disease and a premalignant condition of the oral cavity. Of late, a renewed interest in the role of copper and zinc as a mediator of fibrosis in OSMF has been postulated. Hence; we planned the present study to assess the role of mucosal copper and zinc levels in OSMF patients. **Materials & methods:** The present study was carried on the patients that were diagnosed histopathologically as Oral Submucous Fibrosis (OSMF). A total of 40 cases of OSMF and 20 cases of without any lesion and habit (normal control) were included in the present study. The preserved tissue samples were weighed initially and an aliquot was prepared by dissolving it in 1ml concentrated nitric acid. This aliquot was then diluted with 2 ml deionized water. For the copper estimation a dilution in the ratio of 1:1 of the solution was used and a ratio of 1:5 for zinc estimation. Estimation of the copper and zinc content was achieved by the colourimetric method. The absorbance of these samples was compared to that of the standard solution provided in the kits at 578 nm in a digital photometer. All the results were analyzed by SPSS software. **Results:** While comparing mean mucosal copper and zinc levels in between OSMF patients and normal controls, we obtained significant results. **Conclusion:** Crucial role is played by copper and zinc in the pathogenesis of OSMF.

Key words: Copper, Mucosal, Oral submucous fibrosis, Zinc

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INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic debilitating disease and a premalignant condition of the oral cavity. It is characterized by inflammation and progressive generalized submucosal fibrosis, leading to limitation of mouth opening.¹ It exhibits characteristic histological features consisting of juxtraepithelial hyalinization and excessive collagen deposition in the connective tissue, secondary to which the epithelium becomes atrophic.²⁻⁴ The possible precancerous nature of OSMF was first described by Paymaster, who observed the onset of slow-growing squamous cell carcinoma in one third of such patients. It is considered as a premalignant stage of oral cancer, and the reported risk of malignant transformation varies from 7-13%. The pathogenesis for malignant transformation is not precisely known and is thought to be multifactorial. The carcinogenicity of tobacco in synergism with areca nut is well known, but recently, the carcinogenicity of areca nut alone without tobacco has also been identified.⁵⁻⁸ Of late, a renewed interest in the role of copper and zinc as a mediator

of fibrosis in OSMF has been postulated.⁹ Hence; we planned the present study to assess the role of salivary copper and zinc levels in OSMF patients.

MATERIALS & METHODS

The present study was carried on the patients that were diagnosed histopathologically as Oral Submucous Fibrosis (OSMF) in Jammu region. Ethical clearance was taken from the Ethical Committee of the Dental College and Hospital and written informed consent was obtained from all the subjects. Subjects suffering from chronic systemic illness and those consuming areca nut (in control group) were excluded. Inclusion criteria comprised subjects diagnosed with OSMF (in the study group). A total of 40 cases of OSMF and 20 cases of without any lesion and habit (normal control) were included in the present study. The preserved tissue samples were weighed initially and an aliquot was prepared by dissolving it in 1ml concentrated nitric acid. This aliquot was then diluted with 2 ml deionized water. For the copper estimation a dilution in the ratio of 1:1 of the solution was used and a ratio of 1:5 for zinc estimation.

Table 1: Showing the Mean values & Standard Deviation of the mucosal copper concentration ($\mu\text{g/gm}$) observed in Normal Control (NC) and patients of OSMF

GROUPS	NO. OF SPECIMENS	MUCOSAL COPPER LEVELS	
		MEAN	S.D
NC	20	2.95	0.75
OSMF	40	4.82	0.40

Table 2: Showing the Mean values & Standard Deviation of the mucosal Zinc concentration ($\mu\text{g/gm}$) observed in Normal Control (NC) and patients of OSMF.

GROUPS	NO. OF SPECIMENS	MUCOSAL ZINC LEVELS	
		MEAN	S.D
NC	20	34.58	3.95
OSMF	40	25.64	4.02

Table 3: Showing the t-value and p-value of the mean mucosal copper levels comparing between specimens of OSMF and specimens of normal control.

GROUPS	No. OF SPECIMENS	MEAN MUCOSAL COPPER LEVELS	
		t - value	p - value
OSMF vs NC	60	6.51	<0.05 (s)

S = Significant

Table 4: Showing the t-value and p-value of the mean mucosal zinc levels comparing between 40 specimens of OSMF and 20 specimens of normal control.

GROUPS	No. OF SPECIMENS	MEAN MUCOSAL ZINC LEVELS	
		t - value	p - value
OSMF vs NC	60	7.51	<0.05 (s)

S = Significant

Estimation of the copper and zinc content was achieved by the colourimetric method. The absorbance of these samples was compared to that of the standard solution provided in the kits at 578 nm in a digital photometer. All the results were analyzed by SPSS software. Student t test was used for assessment of level of significance.

RESULTS

By evaluating the mean mucosal copper and zinc levels in between OSMF patients and normal controls, we obtained significant results.

DISCUSSION

In the present study, we observed that significant results were obtained while comparing mean mucosal copper and zinc levels in between OSMF patients and normal controls. Shetty et al evaluated the levels of copper, zinc and iron in saliva of patients with oral leukoplakia, oral submucous fibrosis and oral squamous cell carcinoma. There was a highly significant increase in the level of salivary copper in oral submucous fibrosis patients when compared to controls ($P = 0.001$). Salivary copper levels were also elevated in oral leukoplakia and oral cancer patients ($P = 0.01$). There was a significant decrease in the salivary zinc levels in all three study groups when compared to controls ($P = 0.001$). A highly significant reduction in salivary iron levels was noticed oral submucous fibrosis group. The copper to zinc

ratio significantly increased in all the study groups when compared to controls. Results suggest that salivary copper zinc and iron could be used as biomarkers for oral precancer and cancer.¹⁰

Singh et al estimated tissue copper level in OSMF patients with habit of areca nut chewing and to correlate any change in tissue copper level with histopathological grading of OSMF. A hospital based case-control study was done on 30 subjects visiting out-patient department (OPD) of Kothiwal Dental College Moradabad, of which 15 were clinically diagnosed OSMF cases with habit of areca nut chewing and 15 were taken as controls with no habit of areca nut chewing. Tissue copper levels were measured by Atomic Absorption Spectrophotometer (AAS). The study showed highly significant difference in mean tissue copper level ($P < 0.001$) in patients with OSMF and controls, with patients exhibiting higher tissue copper level (6.43 ± 1.11) in contrast to control who presented low tissue copper level (4.35 ± 0.91), also a highly significant correlation ($P < 0.001$) was seen between increase in tissue copper level and histopathological staging of OSMF. The present study confirms the hypothesis that copper level is increased in areca nut chewers presenting OSMF. Moreover, copper level increased with increased in histopathological grade of OSMF.¹¹ Dey et al evaluated the salivary Cu and Zn levels in OSMF patients. They evaluated a total of 60 patients and divided them into two groups; Group A consisted of 30

patients that were histologically diagnosed with OSMF. Group B consisted of patients that were control and didn't have any oral lesion. Salivary Cu and Zn levels were estimated. All the results were analyzed by SPSS software. Chi square test were used for the assessment of level of significance. Mean salivary Cu levels in OSMF and normal control patients were 0.087 and 0.055 respectively. Mean salivary Zn levels in both OSMF and normal control patients were 0.102. Significant alterations were observed while comparing the mean salivary Cu levels in the two study groups while comparing the mean salivary Zn levels, no significant alterations was seen. Salivary markers can be used for predicting the prognosis and diagnosis of the disease.¹²

CONCLUSION

From the above results, the authors concluded that crucial role is played by copper and zinc in the pathogenesis of OSMF.

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