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Original Research

Oral Submucous Fibrosis: Correlation of Cytological Staining of Copper with Histopathological Grading

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ABSTRACT:
Background: Oral submucous fibrosis(OSF) is a well recognized potentially malignant condition of the oral cavity. There is strong evidence associating the disease with the habit of areca nut. The copper content in areca nut products to be relatively high and it is released in the mouth while chewing. The addition of copper to fibroblasts at concentrations compatible with that found in saliva after chewing areca nut cause a significant increase in the synthesis of collagen. There are very few studies were carried out to determine the copper levels in buccal epithelial cells of patients with oral submucous fibrosis. **Aim:** The following study was carried out to determine the copper levels in buccal epithelial cells of patients with oral submucous fibrosis and controls to throw light on role of copper in etiopathogenesis of OSF. **Materials and Methods:** The study was carried out on 40 patients of oral submucous fibrosis and 10 normal healthy controls with comparable age and sex and were clinically categorized into three clinical stages according to their ability to open the mouth. Two cytological smears were taken from both OSF patients and control and staining of the smear was done by a modified rhodanine technique. The slides were examined under light binocular microscope and the patterns of staining were classified into negative, mild, moderate and intense. **Results:** The control smears showed pale red stain within cytoplasm of all epithelial cells. Out of 40 patients of oral submucous fibrosis, 13(32.5%) patients showed mild copper content, 15(37.5%) patients showed moderate copper content and 12(30%) patients showed intense copper content in buccal epithelial cells. From them 33 patients of clinical grade II, 13(39.39%) patients showed mild copper content, 14(42.42%) patients showed moderate copper content and 6(18.18%) patients showed intense copper content in buccal epithelial cells. Out of 7 patients of clinical grade III, 1(14.28%) patient showed moderate copper content and 6(85.71%) patients showed intense copper content in buccal epithelial cells. **Conclusion:** The correlation of clinical grade with copper content in exfoliated buccal epithelial cells was significant (P=0.002).
Key words: Oral submucous fibrosis, copper, areca nut, oral exfoliative cytology.

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INTRODUCTION

Oral submucous fibrosis (OSF) is a premalignant disorder associated with the chewing of areca nut (betel nut). It is characterized by a mucosal rigidity of varying intensity due to the fibroelastic changes of the juxtaepithelial layer, resulting in a progressive inability to open the mouth. This disease is prevalent in Indian and South Asian population.[1,2,3] OSF causes significant morbidity with Malignant transformation into squamous cell carcinoma (SCC), rate as high as 7.6% in Indian subcontinent.[4]

It has been reported that OSF occurs mainly in persons who are habituated to chew areca nut or products containing arecanut along with other ingredients. In the

recent years with the introduction of commercially available Gutkha and other areca nut products, the incidence of OSF is increasing especially in the younger generation. [5,6,7] Arecanut has shown to have high copper content compared to other commonly eaten nuts. Chewing areca nut for 5 – 30 minutes significantly increases soluble copper in whole mouth saliva. [8] Its been demonstrated that addition of copper to fibroblasts at concentrations compatible with that found in saliva after chewing areca nut cause a significant increase in the synthesis of collagen. [9]

Ma et al discovered increased lysyl oxidase activity in fibroblasts cultured from OSF patients. Lysyl oxidase, an extra cellular copper enzyme is secreted by fibroblasts and initiates cross linking of collagen. [10]

The following study was carried out to determine the copper levels in buccal epithelial cells of patients with oral submucous fibrosis and controls to throw light on role of copper in etiopathogenesis of OSF.

MATERIAL AND METHODS:

The study was carried out on 40 patients of oral submucous fibrosis who attended the Oral Diagnosis and Radiology department of Government Dental College and Hospital, Ahmedabad. All the patients had not received any kind of treatment for oral submucous fibrosis and none of them were suffering from any systemic diseases.

10 normal healthy controls with comparable age and sex were selected in the age range of 20 to 40 years. The controls had no pernicious habits of areca nut chewing or consumption of tobacco in any form and had normal healthy looking mucosa. They were not suffering from any systemic diseases. After taking the history of each patient in the proforma, OSF cases were clinically categorized into three clinical stages according to their ability to open the mouth as given below:

Stage I - Mouth opening ≥ 45mm
Stage II - Mouth opening 20-44mm
Stage III - Mouth opening ≤ 20mm

Cytological staining: Two cytological smears were taken from both OSF patients and control after the oral mucosa was dried with a gauze swab to remove surface debris and excess saliva. Smears were taken from the lesion in buccal mucosa of each individual using a wet wooden spatula, and transferred to clean, dry marked glass slides. They were then immediately fixed with a carbowax fixative. After proper fixation, The Modified Rhodanine technique (Lindquist method 1969)[11] using 5-p DMAB rhodanine- 97% pure (Sigma Aldrich) as the principle reagent was used for staining the cytological smears for the purpose of estimating copper in the buccal mucosal smears.

Microscopic evaluation of stained cytological smear: The slides were examined under light binocular microscope and in each slide one hundred cells were evaluated. The patterns of staining were classified into negative, mild, moderate and intense.

RESULTS:

On evaluation we observed that the control smears which were dipped in supersaturated copper sulfate solution showed pale red stain within cytoplasm of all epithelial cells **(Figure: 1)**. Out of 40 patients of oral submucous fibrosis, 13(32.5%) patients showed mild copper content **(Figure: 2)**, 15(37.5%) patients showed moderate copper content **(Figure: 3)** and 12(30%) patients showed intense copper content **(Figure: 4)** in buccal epithelial cells. From them 33 patients of clinical grade II, 13(39.39%) patients showed mild copper content, 14(42.42%) patients showed moderate copper content and 6(18.18%) patients showed intense copper content in buccal epithelial cells. Out of 7 patients of clinical grade III, 1(14.28%) patient showed moderate copper content and 6(85.71%) patients showed intense copper content in buccal epithelial cells.

These findings suggested that correlation of clinical grade with copper content in exfoliated buccal epithelial cells was significant (P=0.002). (Table: 1)

Figure 1: Smear obtained from non chewers showing absence of copper content in buccal epithelial cells.(40x)

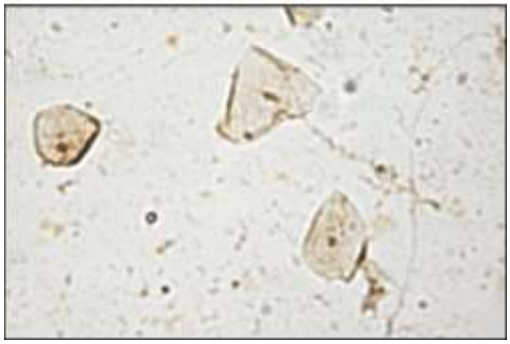

Figure 2: Smear obtained from OSF patient showing mild copper content in buccal epithelial cells.(40x)

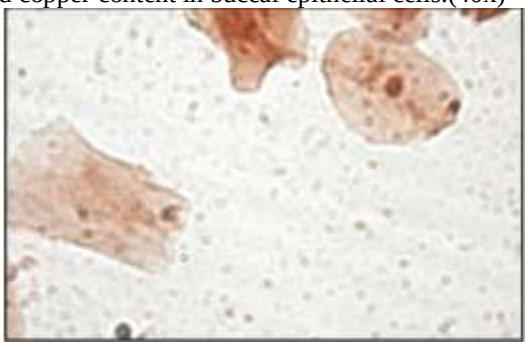

Figure 3: Smear obtained from OSF patient showing moderate copper content in buccal epithelial cells.(40x)

Figure 4: Smear obtained from OSF patient showing intense copper content in buccal epithelial cells.(20x)

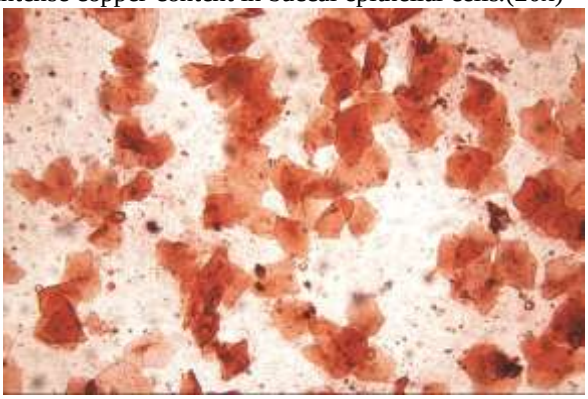

TABLE 1: CORRELATION OF CLINICAL GRADE WITH COPPER CONTENT IN EXFOLIATED BUCCAL CELLS.

Clinical Grade	Total	Copper Content		
		Mild	Moderate	Intense
Grade I	_	_	_	_
Grade II	33	13(39.39%)	14(42.42%)	6(18.18%)
Grade III	7	_	1(14.28%)	6(85.71%)
Total	40	13(32.5%)	15(37.5%)	12(30%)

DISCUSSION

Oral submucous fibrosis is a well recognized potentially malignant condition of the oral cavity. The disease is prevalent among the south Asian countries. Various factors have been implicated in the pathogenesis but there is strong epidemiological evidence associating the disease with the habit of areca nut. [1,2,3]

Trivedy et al (1997) demonstrated that areca nut contains a high copper content and that areca nut chewing may raise salivary levels of soluble copper. There has been a recent interest in the role of copper as a possible etiological factor in the development of OSF. [8]

In the present study, 14(42.42%) patients with clinical grade II, showed moderate copper content in buccal epithelial cells while 6(85.71%) patients with clinical grade III, showed intense copper content in buccal epithelial cells. The association between clinical grade of OSF and copper content in buccal epithelial cells was significant (p value = 0.002). Rooban et al (2004) carried out similar study in non chewers, chewers and OSF patients and they found intense staining pattern in OSF patients but they had not correlated these findings with clinical stage of OSF. [12] Trivedy et al (1997) determined the soluble concentration of copper in whole mouth saliva which peaked after chewing pan masala for 10 to 20 minutes and dropped to control values 10 minutes after chewing stopped. This allows the high copper content of the mixture to remain in contact with the tissue there by possibly increasing the chances of local absorption. [8] Trivedy et al (2000) reported that copper reach to the connective tissue by transmucosal transport through the epithelial cells probably bounded to metallothionein protein, by non energy dependent diffusion[9]

In the present study, variation in intensity, appearance of copper content in buccal epithelial cells of OSF patients could be due to the changes in the permeability of the epithelium. The changes could be either due to the disease process itself i.e. atrophic epithelium or the alteration in the metallothenin that transports the copper into the cell or change in the spatial arrangement of copper with the cell.

It was suggested by Ma et al (1995) that there is an up regulation of the copper dependent extra cellular enzyme lysyl oxidase which stimulate fibroblasts in OSF leading to excessive cross linking of collagen there by inhibiting its degeneration and causing accumulation. In their study lysyl oxidase activity was found to be increased in cultured fibroblasts from OSF patients than in normal controls, although they resemble in many other ways. Collagen which is cross linked by lysyl oxidase was found to be 10 times more resistant to digestion by mammalian collagenase. [10] Subsequently Trivedy et al (1997) in their study confirmed these findings. [8]

Its been reported that Significantly raised copper content in buccal epithelial cells of OSF patients have

important implication in the aetiopathogenesis of this condition and its progression to malignancy because copper may induce DNA damage and alter the p53 conformation[9].

CONCLUSION:

As our results show that the correlation of clinical grade with copper content in exfoliated buccal epithelial cells was significant. Hence our study supports the role of copper in the pathogenesis of OSF.

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