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Analysis of copper and zinc levels in the mucosal tissue and serum of oral submucous fibrosis patients

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***Abstract*---**Background and Objectives:Although the pathogenesis of oral submucous fibrosis (OSMF) has been studied, it remains unclear whether or not this illness might progress to malignancy. The pathogenesis of the illness may be better understood by measuring copper and zinc levels in tissue and serum from these people. Because of the widespread cultural and social usage of areca nuts in India, a research was conducted to investigate their concentrations in this population. Methods:Twenty people with OSMF and twenty people without it were studied in a case-control fashion. Copper and zinc concentrations were measured in the 40 patients' tissues and blood using colorimetry. Compared to the zinc concentration of 25.18 4.92 g/gm found in OSMF group tissues, copper concentration was 4.31

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1.13 g/gm. Zinc levels were 0.92 0.18 g/ml and serum copper levels were 1.00 0.20 g/ml on average in the OSMF group. Interpretation and conclusion:OSMF group tissue zinc levels were substantially different from control group levels (p = 0.009), as were OSMF group tissue copper levels (p = 0.001). There was no statistically significant difference in serum copper (p = 0.35) or zinc (p = 0.08) between the experimental and control groups. These data provide further support for the hypothesis that oral mucosal copper and zinc play a role in OSMF pathogenesis.

***Keywords*---**copper, zinc, oral submucous fibrosis, trace elements.

Introduction

The oral ailment known as oral submucous fibrosis (OSMF) is a serious one that may develop into cancer. Aware monitoring is required since it may lead to oral cancer, a lethal illness that is increasing in both younger and older populations. Interventions in high-risk individuals are necessary in poor nations to prevent the catastrophic and widespread effects of oral cancer, preferably before the illness becomes invasive but at the very least before it advances locally or metastatically. [1] The ancient saying, "Prevention is better than cure," is as relevant now as it ever was. To effectively combat oral cancer, screening for and treating precancerous lesions is currently the most promising strategy. Areca nut eating has been linked to an increased risk of developing occupational snuffbox disease (OSMF). While the number of people diagnosed with oral submucous fibrosis has been steadily increasing in recent years, the exact mechanisms that lead to the development of this ailment remain unclear. There is a lot of disagreement on whether or not trace elements have a role in many illnesses, with different writers reporting contradictory findings. [2] There has been a lot of research on trace elements in recent years to see whether they have a role in the genesis of oral malignant diseases. There has been a dearth of research on oral premalignant diseases. [3]The following research was conceived and executed based on the hypothesis that determining copper and zinc levels in the tissue and blood of patients with oral premalignancies may shed light on the pathophysiology, determine the prognosis, and facilitate the successful treatment of these lesions.

Objectives

- In an effort to ascertain if copper and zinc have a major role in the onset of oral submucous fibrosis.
- Examining the potential of copper and zinc levels as predictive indicators of oral submucous fibrosis is the focus of this investigation.
- Specifically, we want to determine if serum or tissue provides a more appropriate medium in which to evaluate these biochemicals.

Methodology

Case-control research was conducted at Dental College. Forty patients were included in the research, 20 of them were randomly assigned to each of two

groups based on their reasons for visiting the dental clinic. Group A consisted of patients having a clinical diagnosis of oral submucous fibrosis (OSMF), whereas Group B was composed of healthy individuals without any history of disease or oral habits that may be linked to areca nuts (Group B). Group A was clinically diagnosed with oral submucous fibrosis according to the specified criteria3 and then staged as I, II, or III. Patients with other oral mucosal problems, those with systemic illnesses, those who had taken copper or zinc supplements in the preceding year (orally or parenterally), and those who had previously had therapy for oral submucous fibrosis were not included in the research.

After receiving written informed permission, all patients meeting the aforementioned criteria were recruited in the research. After participants were enrolled, researchers interviewed them extensively to collect data on their usage of areca nuts, paan, and gutkha (commercially available processed areca nut preparations), including the length of time they had used each product and the frequency with which they used it. Prior to the removal of impacted third molars or the removal of pericoronal flaps, biopsies were taken under local anesthesia from representative areas of the oral mucosa in the cases and the retromolar region in the controls. Standard aseptic procedures were used to extract 5 ml of venous blood from the median cubital vein to get the serum. Tissue and serum were frozen so that they might be used later in research. The tissue samples were weighed and then dissolved in 1 ml of strong nitric acid to create an aliquot. Totaling 2 ml, deionized water was added to this aliquot. For the copper estimate, the solution was diluted 1:1; for the zinc estimate, it was diluted 1:5. Specifically, blood samples were diluted 1:1 with deionized water and 1:5 with distilled water such that copper and zinc concentrations could be determined after being stored. We were able to estimate the copper and zinc concentrations using a colorimetric method. Copper and zinc estimate kits (Crest Biosystems, Goa, India) included all of the necessary components for performing the analysis, including tissue and blood samples that were combined with the required amounts of buffer and color reagents. Using a digital photometer set to measure absorbance at 578 nm, we compared the samples to the standard solution included in the kits (PhotochemMicrodigital Colorimeter, AIMIL, India). The outcomes of the surgeries were recorded and examined statistically (Mean, Standard deviation, Mann-Whitney U test).

Results

Participants' ages varied from 22 to 60 years old, with the majority (65%) being in the millennial demographic. Almost all of them were men (19/20), making nearly 95% of the total. Patients who reported utilizing items containing areca nuts often chewed "paan," or betel quid, which is made by wrapping raw areca nut and slaked lime in the leaf of the vine piper betel. Sixty percent of the participants in this study chewed gutkha, while the other forty percent used the more conventional paan or betel quid. In group A, the average tissue copper concentration was found to be 4.31 1.13 g/g, whereas the average tissue zinc concentration was 25.18 4.92 g/g. It was found that the average serum copper level in lot A was 1.00 0.20 g/ml, whereas the average serum zinc level was 0.92 0.18 g/ml. Average tissue copper concentration in group B was 2.89 0.36 g/g, whereas zinc fixation averaged 32.46 3.79 g/g. Overall, this healthy population

had a serum zinc fixation of 0.98 g/ml and a blood copper concentration of 1.11 g/ml (Figs 1 and 2). We found that the average levels of copper and zinc in the tissues of people who smoked gutkha were 4.81 and 25.69 micrograms per milligram, respectively, whereas those of those who smoked paan were 3.56 and 24.91 micrograms per milligram, respectively (Table 1). During the three clinical phases, patients' mean tissue and serum copper and zinc levels changed (Table 2). Based on a statistical analysis of the data from both groups we found: The results showed a significantly significant (p 0.001) variation in tissue copper levels. When comparing zinc concentrations in different tissues, a huge difference was found (p = 0.009). Neither the serum zinc nor the blood copper levels changed significantly (p = 0.35 and p = 0.08, respectively).

Table 1
Influence of habits on mean values of copper and zinc in the cases

	Element	*Habit*	*n*	*Mean*	*Standard deviation*
Tissue (tg/g)	Copper	Gutkha	11	4.79	0.81
		Paan	9	3.52	1.11
	Zinc	Gutkha	11	25.64	3.77
		Paan	9	24.86	6.23
Serum (tg/ml) Copper	Copper	Gutkha	11	0.92	0.18
		Paan	9	1.01	0.21
	Zinc	Gutkha	11	0.83	0.11
		Paan	9	1.00	0.22

Table 2
Influence of clinical stages on mean values of copper and zinc

Element		*Clinical stage*	*n*	*Mean*	*Standard deviation*
Tissue (tg/g)	Copper	Stage I	4	3.12	0.95
		Stage II	12	4.56	0.85
		Stage III	4	4.66	1.23
	Zinc	Stage I	4	28.34	7.16
		Stage II	12	23.45	3.65
		Stage III	4	25.37	2.56
Serum (tg/ml)	Copper	Stage I	4	1.01	0.21
		Stage II	12	0.89	0.20
		Stage III	4	1.03	0.06
	Zinc	Stage I	4	0.78	0.21
		Stage II	12	0.87	0.16
		Stage III	4	0.76	0.12

Discussion

The purpose of this study was to analyse copper and zinc concentrations in the participants' blood serum and oral mucosal tissue. Trace element levels were obtained by randomly assigning patients to one of two groups of twenty. Among the findings of this research is the fact that the majority of patients in group A (OSMF Group) (65%) were between the ages of 21 and 30. While a case control study in the same age range found 46.2% in Delhi, this number is higher. [4] The ratio of men to girls in group A was 19 to 1. (males:females). This preponderance of males is consistent with findings in previous research. [4-6] These writers have noticed that women in India are less likely to engage in oral chewing due to societal norms. Even though these behaviors are socially frowned upon, there seems to be more tolerance of them among men than among women. This likely increases the likelihood that men will get addicted to these behaviors and exhibit OSMF. Group A patients reported utilizing commercially available processed gutkha at a rate of 60%. This is in contrast to the previously published data showing that 38.5% of gutkha chewers and 52.2% of betel quid/ paan chewers4, but it is in line with the data showing that 71.0% of gutkha chewers.

In comparison to the control group, group A had considerably higher mean copper tissue levels. To a lesser degree, this is in line with the finding that copper levels were higher than normal in OSMF tissues (albeit not significantly so). [7] Copper levels in that study's group (n = 11) were found to be higher, at 5.5 2.9 g/g, but no significant correlation was identified between the two investigations. As a result, it is possible that this is because their research group was too small. Additionally, our study's controls had a mean tissue copper value of 2.89 0.36 g/g, which was lower than the 41.9 g/g discovered in the aforementioned study's controls. [7] A possible explanation for the discrepancy in copper levels between the two investigations is that the individuals in the former ate more areca nut-related items than those in the latter. Copper discharged from the areca nut product while chewing would come into direct touch with the oral mucosal keratinocytes, it has been pointed out. [7] Copper linked to metallothionein is likely taken up into epithelial cells without using cellular energy. To that end, it is reasonable to assume that copper plays a key role as a catalyst in OSMF. Copper stimulated oral fibroblasts, according to an in vitro study[8], by increasing expression of lysyl oxidase.

Cytological analysis confirmed that OSMF patient smears showed more intense staining compared to non-chewers' smears, lending support to the hypothesis that copper had a role in the genesis of OSMF. [9] Due to their well-established metabolic interdependence, copper and zinc both play essential roles. [10] Zinc is associated with a decrease in copper absorption because of its role in controlling mucosal metallothionein levels. Therefore, we attempted to calculate the zinc concentrations in OSMF patients' tissues in the current investigation. Generally, zinc concentrations in tissues were measured to be 25.18 4.92 g/g on average. As compared to the levels seen in the control group, where zinc levels were 32.46 3.79 g/g, this was a huge improvement. Therefore, this discovery provides more evidence in support of the hypothesis that a rise in copper prices would be accompanied by a corresponding fall in zinc prices. 10 The effects of lifestyle factors on copper and zinc levels in Group A were evident in both tissue and blood

samples. Average values were greater for gutkha consumers than for paan consumers, suggesting that the refined version of areca nut was more harmful than the unrefined raw kind. There was a consistent increase or decrease in the mean tissue values for the two components throughout all three clinical periods in Group A. Thus, as time went on, so did the concentrations of these components in the tissue. Because of this, the two biochemicals have been proposed as potential prognostic markers for ocular schwannoma. However, there was no significant difference between group A and control findings for the trace element concentrations in the serum. Evidence like this suggests that copper and zinc concentrations and interactions occur locally inside the oral mucosa, rather than being compounded by systemic changes, is very significant. Our results show that copper levels rose and zinc levels dropped in OSMF tissues, supporting the involvement of these biochemicals in the pathogenesis of oral submucous fibrosis. In addition, our findings recommend using oral mucosal tissue rather than blood to evaluate these biochemical trace components when investigating oral submucous fibrosis.

Conclusion

The data was analysed statistically, and the results were interpreted to lead to the following conclusions concerning copper concentrations in the oral mucosa and a noticeable drop in tissue zinc content. This evidence suggests that both increased copper and decreased zinc act locally in OSMF tissue. The effects of OSMF on serum copper and zinc levels are theoretical but unlikely to be functionally significant. For this reason, measuring copper and zinc levels in oral mucosal tissue rather than serum is preferable in people with OSMF. Several factors, including changes in chewing behaviour and elevated copper and decreased zinc levels in tissues, have been related to OSMF's three clinical stages. The more refined the gutkha, the greater the copper and lower the zinc levels in the tissue. Prognostic indications based on biochemical markers have been shown to be useful in assessing the likelihood of OSMF.n

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