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RESEARCH ARTICLE

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: Oral submucous fibrosis (OSMF) is a well-recognized potentially malignant disease of oral mucosa whose exact pathogenetic mechanisms have not been understood. Measurement of copper and zinc levels in the tissue and serum of these patients may be helpful in understanding the pathogenesis. Therefore, a study was undertaken to analyze the levels of these trace elements in the populations of the coastal region of south Karnataka and north Kerala, India, who show predominant use of areca nut due to their cultural and social backgrounds.

Methods: A case-control study was conducted on 20 cases of OSMF and 20 controls. The tissue and serum concentrations of copper and zinc in these 40 subjects were measured by colorimetry.

Results: The mean tissue copper level in OSMF group was 4.31 ± 1.13 µg/gm, while the tissue zinc level was 25.18 ± 4.92 µg/gm. The mean serum copper level in the OSMF group was 1.00 ± 0.20 µg/ml while the mean serum zinc level was 0.92 ± 0.18 µg/ml.

Interpretation and conclusion: The tissue copper levels in OSMF group showed very highly significant difference (p = 0.001) from the controls while the difference in tissue zinc levels was highly significant (p = 0.009). The serum levels of copper (p = 0.35) and zinc (p = 0.08) did not exhibit any statistical difference from those of the control group. These findings indicate that the trace elements, copper and zinc, within the oral mucosa definitely, have a role to play in the pathogenetic mechanisms of OSMF.

Keywords: Copper, Zinc, Oral Submucous fibrosis, Trace elements.

INTRODUCTION

Oral submucous fibrosis (OSMF) is a well-recognized potentially malignant condition of oral cavity. It can lead to oral cancer, a disfiguring and potentially fatal disease continuing to rise in incidence among younger and older people alike necessitates astute surveillance. In developing countries, controlling the devastating, widespread consequences of oral cancer requires interventions in at-risk persons ideally before the disease becomes invasive but certainly before it becomes locally advanced or metastatic.[1] The age-old adage—"*Prevention is better than cure*" still holds true in the contemporary world. Therefore, detection of the premalignancies and preventing them from malignant transformation seems to be the best available tool in the fight against oral cancer. OSMF has been definitively associated with the habit of chewing areca nut. In recent times, though there has been a consistent rise in the number of patients with oral submucous fibrosis, there is still no detailed understanding of the mechanism directly delineating the aetiopathogenetic mechanism involved in the formation of condition.

The role of trace elements in various diseases has been a matter of controversy with various authors reporting on conflicting data.[2] Trace elements have been extensively studied in recent years to assess whether they have any modifying effects in the aetiology of oral malignant conditions. Relatively less scientific work has been performed in the area of oral premalignant conditions. With the scientific premise in mind that the measurement of copper and zinc levels in the tissue and serum of the patients with oral premalignancies may be helpful in understanding the pathogenesis, establishing the prognosis and rendering effective treatment of these lesions, the following study was designed and conducted.

OBJECTIVES

- To establish whether copper and zinc have any role to play in the pathogenesis of oral submucous fibrosis.
- To evaluate whether copper and zinc could be used as prognostic indicators in the development of oral submucous fibrosis.
- To determine whether serum or tissue is a better medium for evaluating these two biochemicals.

METHODOLOGY

A case control study was designed and carried out in Yenepoya Dental College, Mangalore, India. The study consisted of totally

40 patients, who visited the dental clinic for various reasons and were divided into two groups of 20 patients each. The cases were clinically diagnosed as suffering from oral submucous fibrosis (OSMF) (Group A) and the control group with no apparent lesions of the oral mucosa and without any areca nut related oral habits (Group B). The clinical diagnosis of oral submucous fibrosis in Group A was made by using the described criteria[3] and classified into three clinical stages – Stage I, II and III. Patients manifesting any other oral mucosal conditions, suffering from any systemic diseases, with a history of any copper or zinc supplementation in any form, oral or parenteral, in the past one year or those having undergone any previous treatment for oral submucous fibrosis were excluded from the study.

All the patients fulfilling the above criteria were enrolled in the study after obtaining written informed consent. All the enrolled subjects were then subjected to a detailed interview with emphasis on recording any oral habits of chewing areca nut, paan (betel quid), and gutkha (commercially available processed areca nut preparations), the duration of habit practice, the frequency of each habit per day. An incisional biopsy was performed under local anesthesia from a representative part of the oral mucosa in the cases and from the retromolar area of the controls, who were undergoing impacted 3rd molar extraction or pericoronal flap removal. By venipuncture of the median cubital vein under aseptic precautions, 5 ml of venous blood was obtained, subsequently from which serum was obtained. The tissue and serum obtained were preserved in a frozen state until analysis.

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. The preserved serum samples were diluted with deionized water in a ratio of 1:1 for copper estimation and a ratio of 1:5 for zinc estimation.

Estimation of the copper and zinc content was achieved by the colourimetric method. The tissue and serum samples used for the estimation were mixed with appropriate proportions of buffer and color reagents supplied in the copper and zinc estimation kits (Crest Biosystems, Goa, India) as per the manufacturer's instructions. The absorbance of these samples was compared to that of the standard solution provided in the kits at 578 nm in a digital photometer (Photochem Microdigital Colorimeter, AIMIL, India). The data obtained from the procedures were tabulated and analyzed using statistical methods (Mean, Standard deviation, Mann-Whitney U test).

RESULTS

In the study group the age of the subjects ranged from 22 to 60 years, with a major share (65%) of the cases were within 21 to 30 years. Males comprised 95% of this group (19/20). All the patients reported chewing habits related to areca nut and they either chewed 'paan' or betel quid made by rolling raw areca nut and slaked lime in the leaf of the vine piper betel, or used commercially available processed mixture of areca nut and tobacco called 'gutkha/paan masala'. It was observed that majority of subjects in this group were gutkha chewers (60%), while the remaining (40%) chewed the traditional form of paan/betel quid.

The mean tissue copper level in group A was calculated to be 4.31 ± 1.13 µg/gm, while the tissue zinc level was 25.18 ± 4.92 µg/gm. The mean serum copper level in group A was calculated to be 1.00 ± 0.20 µg/ml, while the mean serum zinc level was 0.92 ± 0.18 µg/ml. The mean tissue copper level in group B was found to be 2.89 ± 0.36 µg/gm, while the tissue zinc level was 32.46 ± 3.79 µg/gm. The mean serum copper level in this control group was 1.11 ± 0.32 µg/ml, while the mean serum zinc level was 0.98 ± 0.16 µg/ml (Figs 1 and 2).

The mean tissue copper and zinc levels in group A subjects chewing gutkha was 4.81 ± 0.85 µg/gm and 25.69 ± 3.93 µg/gm respectively, and in subjects chewing paan, it was 3.56 ± 1.13 µg/gm and 24.91 ± 6.35 µg/gm respectively (Table 1). The mean tissue and serum levels of copper and zinc were noted to be different among the patients with three clinical stages (Table 2).

On statistical comparison between the two groups (Table 3), the following findings were obtained: difference in tissue copper

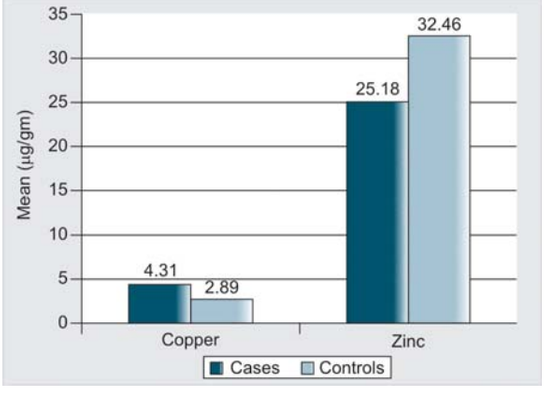

Fig. 1: Mean tissue copper and zinc levels

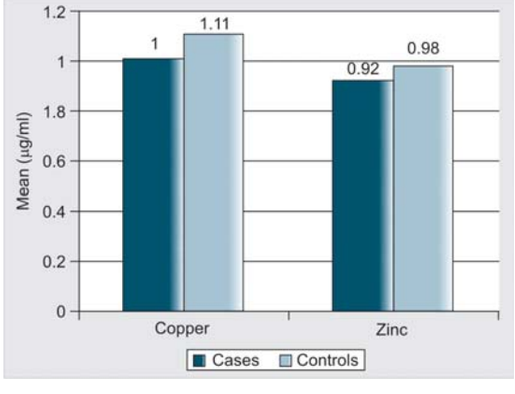

Fig. 2: Mean serum copper and zinc levels

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

	Element	Habit	n	Mean	Standard deviation
Tissue (µg/g)	Copper	Gutkha	12	4.81	0.85
		Paan	8	3.56	1.13
	Zinc	Gutkha	12	25.69	3.93
		Paan	8	24.91	6.35
Serum (µg/ml)	Copper	Gutkha	12	0.97	0.20
		Paan	8	1.05	0.22
	Zinc	Gutkha	12	0.87	0.12
		Paan	8	1.01	0.23

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

	Element	Clinical stage	n	Mean	Standard deviation
Tissue (µg/g)	Copper	Stage I	5	3.18	0.99
		Stage II	11	4.64	0.87
		Stage III	4	4.80	1.20
	Zinc	Stage I	5	28.54	7.24
		Stage II	11	23.57	3.76
		Stage III	4	25.42	2.75
Serum (µg/ml)	Copper	Stage I	5	1.08	0.21
		Stage II	11	0.94	0.22
		Stage III	4	1.07	0.09
	Zinc	Stage I	5	0.96	0.23
		Stage II	11	0.96	0.17
		Stage III	4	0.80	0.14

Table 3: Statistical comparison of the two study groups

	Tissue		Serum	
	Copper	Zinc	Copper	Zinc
Z	3.829	2.850	– 0.930	– 0.247
p value	0.001* (vhs)	0.009* (hs)	0.35* (ns)	0.08* (ns)

Mann-Whitney U Test used.
*$p \leq 0.001$ (Very highly significant), $p \leq 0.01$ (Highly significant), $p > 0.05$ (Not significant)

levels were found to be very highly significant (p = 0.001). The tissue zinc levels showed a highly significant difference (p = 0.009). The serum copper as well as serum zinc levels did not exhibit any statistically significant difference (p = 0.35) and (p = 0.08) respectively.

DISCUSSION

In this study, an attempt was made to analyze the concentrations of trace elements, copper and zinc, in the oral mucosal tissue and serum of the enrolled subjects. The study was performed by dividing the patients into two groups of 20 patients each and the trace element levels were obtained.

Analyzing the results of this study, it can be noted that in group A (OSMF Group), a majority of the patients (65%) were within 21 to 30 years of range. This figure is higher than the 46.2% occurrence of cases in the same age group in the case control study conducted in Delhi.[4] The gender distribution for group A was 19:1 (males:females). This predominant male occurrence is similar to that seen in other studies.[4-6] These authors have observed that the social restrictions prevalent in India prevent the women, to a certain extent, from indulging in the oral chewing habits. Though these habits are considered a taboo, there seems to be more relaxation of these norms for men and, thus there are more chances that males get addicted to the habit and, therefore manifest OSMF with greater frequency.

A large proportion (60%) of the patients in group A chewed the commercially processed 'gutkha'. This is a deviation to the reported findings of 38.5% of gutkha chewers and 52.2% betel quid/ paan chewers,[4] but in concurrence with the findings of 71% gutkha chewers by another study.[6]

The mean tissue levels of copper in group A were very highly significant different from those of the controls. This is in partial concurrence with the reported finding of raised, but not statistically significant, copper levels in the tissues of OSMF.[7] The finding of mean copper levels in that study group (n = 11) was 5.5 ± 2.9 µg/g, which was higher than the mean level in our study, yet there was no statistical correlation in that study. This could be due to the fewer cases comprising their study group. Also the mean tissue copper value of 2.89 ± 0.36 µg/g for the controls in our study was less than 4 ± 1.9 µg/g found in that study.[7] This difference in the concentration of copper between the two studies' subjects could be due to the regional variation in the dietary habits and may be modified by difference in the availability of areca nut related products. It has been pointed out that copper released during chewing of the areca nut product would be in direct contact with the oral mucosal keratinocytes.[7] The uptake of this copper into the epithelial cell is probably by non-energy dependent diffusion by being bound to metallothionein. Copper, therefore probably acts as an initiating factor in OSMF. An in vitro study[8] found that copper stimulated oral fibroblasts by causing upregulation of the enzyme, lysyl oxidase. The significance of copper in the pathogenesis of OSMF has been confirmed by a cytological study where more intense staining in smears of OSMF patients was found as compared to smears form non chewers, lending support to the theory that copper had a role to play in the pathogenesis of OSMF.[9]

The role of copper cannot be segregated from that of zinc because of the well-elucidated biochemical relatedness.[10] Zinc bears an inverse relationship with copper and has been

implicated in the modulation of mucosal metallothionein, thereby interfering with the absorption of copper. Therefore, in the present study, we estimated the tissue levels of zinc in OSMF patients. The mean tissue level of zinc was found to be 25.18 ± 4.92 µg/g. This was in sharp difference to the mean tissue zinc level of 32.46 ± 3.79 µg/g in the controls, a highly significant difference. Thus, this finding confirms the suspicion that when copper values increase, the zinc values are bound to decrease in an inverse manner.[10]

In Group A, the influence of habits was clearly seen on both the tissue and serum levels of copper and zinc. The gutkha chewers had higher mean values than paan chewers indicating that the processed form of areca nut was more damaging than the unprocessed raw variety. Also the three clinical stages in Group A showed progressive changes in the mean tissue values for the two elements. Thus, with increasing progression, the concentrations of these elements also progressively increased (or decreased) in the tissue. This fact points to the role of the two biochemicals as possible prognostic indicators for OSMF.

However, the mean serum values of the trace elements in group A when compared to those in the controls were not found to be statistically significant. This finding points to the fact that there is likely a local change in concentrations and subsequent interactions of these two elements, copper and zinc, within the oral mucosa without any compounding systemic alterations. It can be clearly seen from our findings that the levels of copper increased significantly and those of zinc decreased significantly in the tissues of OSMF, confirming that both these biochemicals have a role in the pathogenesis of oral submucous fibrosis. Also, our findings indicate that the oral mucosal tissue is a comparatively better medium than serum for the evaluation of these biochemical trace elements in the context of oral submucous fibrosis.

CONCLUSION

After application of statistical analysis to the obtained data and interpretation of the results thus obtained, the following inferences could be drawn from our study: Majority of the patients with OSMF chewed the commercially processed areca nut—gutkha as compared to the traditional form of paan/betel quid chewing. OSMF patients have markedly increased concentrations of copper within the oral mucosa, and a definite reduction in the tissue zinc concentration. This suggests that both an increased copper level and a decreased zinc level operate at the local level in OSMF tissue. The alteration of serum levels of both copper and zinc in OSMF is not of any consequence. Thus, in OSMF patients, oral mucosal tissue serves as a better medium for the evaluation of the trace elements, copper and zinc as compared to serum. The three clinical stages of OSMF can be correlated to the progressive increase in tissue copper levels, and progressive decrease in tissue zinc levels, as also on the basis of the chewing habit. The more processed gutkha induced higher tissue levels of copper and decreased levels of zinc. This highlights the use of these biochemicals as prognostic indicators for OSMF.

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