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Original Article

Dentistry Section

Estimation of the Serum and the Salivary Trace Elements in OSMF Patients

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ABSTRACT

Objectives: Gutkha packets contain trace elements like copper, zinc, iron and magnesium. This study compared the levels of the trace elements in patients with gutkha eating habits with or without oral submucous fibrosis and in healthy patients.

Study Design: A total of 75 patients were included in this study and they were divided into three groups; the individuals with a history of gutkha intake with OSMF, the individuals with a history of gutkha intake without OSMF and apparently healthy individuals without OSMF and without any habits.

Blood and saliva was collected and they were subjected for analysis by using atomic absorption spectrometry and a differential pulse anodic stripping voltmeter.

Results: The results were tabulated and they were subjected to a statistical analysis.

Conclusion: There was a significant difference in the serum Mg and Fe levels between the patients with habits and the normal healthy individuals. A significant difference was observed in the serum zinc levels in the patients with habits with and without OSMF. Altered serum trace element levels are documented in malignant cases and they are considered to be good biomarkers for malignancies. The serum copper and Zn levels and the Cu/Zn ratio in OSMF patients can be considered as the markers which show a susceptibility towards cancer.

Key Words: OSMF, Gutkha, Copper, Zinc, Iron, Magnesium

INTRODUCTION

Oral Submucous fibrosis (OSMF) is known and established to be precancerous. In 2002, the statistics for OSMF from the Indian continent alone was about 5 million people (0.5% of the population of India) [1]. This rise in OSMF in India has been attributed to the increased consumption of Gutkha, of which areca nut is the main ingredient [2], along with detectable levels of trace elements like copper, zinc, iron and magnesium [3].

Considering the role of the trace elements in precancerous and cancerous conditions, and the contents of areca nut, a study was undertaken to estimate the serum/saliva concentrations of the trace elements in patients with gutkha chewing habits with or without the incidence of OSMF.

AIMS AND OBJECTIVES

- The estimation and comparison of the serum and the salivary levels of the trace elements in OSMF patients with gutkha chewing habits, in individuals with a habit of gutkha chewing but who were not suffering from OSMF and in healthy individuals with no habits.
- Evaluation of the role of the trace elements in the aetiopathogenesis of OSMF.

Review of the Literature

The trace elements have been extensively studied in recent years, to assess whether they had any role in the aetiology of cancer. Magnesium and zinc are the elements which have essential roles in the regulation of the cell growth, division and differentiation. High levels of copper have been observed to protect against a chemical induction of tumours [4].

The correlation between serum copper and the stage of the disease has indicated that serum copper might serve as a tumour marker or a tumour antigen, in a study which was conducted by Thorling EB and Thorling K [5].

Khanna S and Karjodkar F [6], in their study, concluded that there was an increase in the levels of CICs in precancer and cancer patients, that there was a gradual increase in the serum Cu levels from the precancer to the cancer patients and that the serum Fe levels were significantly decreased in cancer patients. Fisher GL et al., [7] deduced that the ratio between the serum copper levels and the serum zinc levels may be useful in discriminating between the patients with primary and metastatic osteosarcomas.

Vyas RK et al., [8] concluded that the zinc, serum magnesium and the calcium levels were found to be low in the patients with malignancies and cirrhosis.

Oyama T, Kawamoto T et al., [9] in their study, concluded that a preoperative increase in the Cu/Zn levels predicted the tumour progression better than the changes in the Cu or the Zn levels. Also, the measurement of Cu/Zn was useful for assessing both the extent and the prognosis of the disease in NSCLC patients, which was similar to the measurement of the tumour markers such as CEA. Also, the measurement of Cu/Zn had a prognostic significance.

Rajendran R, Babu KN, Nair KM [10] found high levels of magnesium, cadmium and selenium in OSMF. They also stated that the increased levels of cadium and selenium in OSMF might be a part of a mechanism which was involved in the malignant transformation of OSMF.

MATERIAL AND METHODS

A study was conducted on the patients who attended the hospital OPD. The age group of the patients ranged from 16 to 45 years. The patients were randomly selected and they were divided into three groups.

Group A: 30 individuals with a history of gutkha intake of more than one year, with the occurrence of OSMF.

Group B: 30 individuals with a history of gutkha intake of more than one year, without any evidence of OSMF.

Group C: 15 apparently healthy individuals without OSMF and without habits were taken as the controls.

Inclusion Criteria

- The individuals who gave a positive history of areca nut intake in the form of gutkha.
- The individuals who consumed gutkha for more than one year.
- The criteria for the presence of disease was made on the basis of the clinical inspection, a restricted mouth opening, the presence of fibrous bands and the complaint of a burning sensation.

Exclusion Criteria

- The individuals with a history of the consumption of tobacco in any other form such as cigar, bidi, mawa, etc were eliminated.
- The patients with a history of any systemic disease.
- The patients who gave any history of being treated for the same complaint, in the form of local applications, intra lesional injections or a systemic administration of drugs.
- The patients who had OSMF but had stopped the habit of gutkha chewing.
- Any patient who did not want to be a part of the study.

The informed consents of all the patients who were included in the study were taken. A thorough history was taken from each patient and clinical examinations of all the patients were performed; OSMF was confirmed on the basis of the biopsies and the histopathological reports. 5cc of blood was collected for serum and stimulated saliva (10ml) in the morning session.

To the collected serum and saliva; after quantifying them, 2ml of electronic grade concentrated nitric acid and 1ml of concentrated perchloric acid were added. The mixture was boiled till the acid evaporated and a white residue remained. To this, 0.25% nitric acid was added and the solution was warmed up. On cooling, the solution was transferred to 5ml volumetric flasks and a more dilute solution was added to make up the volume to 5ml .

The digested serum and saliva samples were subjected to an analysis of the trace elements (copper, zinc, iron and magnesium) by using atomic absorption spectrometry and a Differential Pulse Anodic Stripping Voltmeter (DPASV).

RESULTS AND OBSERVATIONS

The data was subjected to a statistical analysis by using mean, standard deviation and the Student's unpaired 't'-test [Table/Fig-1 and 2].

DISCUSSION

In our study, the mean serum copper levels in the Group A. (0.8763) were not significantly increased, as compared to those in Group B. (0.8109) or Group C. (0.8285), and they were statistically insignificant.

The copper which is released during chewing is brought in direct contact with the oral mucosal keratinocytes and it is present in the oral environment. It is dissolved in the whole saliva for a prolonged period (up to 30 minutes). Following chewing, the uptake of copper into the epithelial cells occurs probably by a non energy dependent diffusion, where it is either bound to the proteins (mainly metallothioneins) or transferred across the basement membrane [11]. The absorbed copper appears in the blood stream in as little as 15 minutes after its ingestion. In our study, most of the patients had the habit of chewing and spitting out the contents of gutkha after 20-25 minutes.

The salivary copper levels in our study were increased from Group C (0.0168) to Group B (0.0268) to Group A (0.0338). This was in accordance to the findings of the study of Trivedy C et al., who suggested that the increased copper levels in the saliva enhance the uptake of copper by the tissues and that this increase the levels

	Serum							
	Copper		Zinc		Iron		Magnesium	
Group	Mean (µg/ml)	S.D.	Mean (µg/ml)	S.D.	Mean (µg/ml)	S.D.	Mean (µg/ml)	S.D.
A	0.8763	0.2625	1.7926	0.2625	0.6399	0.3379	16.9382	3.9287
B	0.8109	0.2022	1.0000	0.3536	0.603	0.4561	16.559	3.1375
C	0.8285	0.1379	1.2228	0.5761	0.836	0.1998	15.2189	2.128

[Table/Fig-1]: Mean and Standard deviation of levels of Trace Elements in Serum in Group A, B and C
p=0.004 for Zn between Group A & B = Significant; p=0.004 for Fe between Group A & C = Significant

	Saliva							
	Copper		Zinc		Iron		Magnesium	
Group	Mean (µg/ml)	S.D.	Mean (µg/ml)	S.D.	Mean (µg/ml)	S.D.	Mean (µg/ml)	S.D.
A	0.0338	0.0393	0.4472	0.4111	0.1037	0.1135	6.938	2.557
B	0.0268	0.0221	0.3821	0.2470	0.1064	0.0996	7.7469	3.1621
C	0.0168	0.0080	0.2740	0.1006	0.1589	0.0843	5.6600	1.1750

[Table/Fig-2]: Mean and Standard deviation of levels of Trace Elements in Saliva in Group A, B and C
p=0.01 for Mg between Group A & C = Highly Significant

of copper within the tissues [11]. The increased salivary copper levels come back to before –the-chewing values in about 40 minutes.

Trivedy et al., [12] has also reported on the copper induced mutagenesis through the p53 aberrations in OSMF, which may be critical in the progression of the potentially malignant lesions to squamous cell carcinoma.

The copper which is added at various concentrations in vitro has also been shown to increase the proliferation of the fibroblasts in the culture [12]. Another study which was based on the ultrasound investigations of the visceral organs in OSF patients reported that there was no evidence on the fibrotic changes elsewhere. The faecal copper was also normal, thus suggesting that the copper levels were within the tolerance levels [13]. As the oral mucosa is directly exposed to the copper challenge in the chewers, its effect may well be local. These different growth characteristics may either be due to the direct effects of the ingredients of the areca nut or they may be secondary to the inflammatory factors which are mediated by the areca nut, such as IL-1, TGF- , IGF and EG [14]. There is a definite trend with the cases which have the lesion in the faucial bands (1st stage), which exhibit the lowest serum copper and those in stage 3 (the faucial, labial and the buccal bands), which present the highest copper levels in the serum. Hence, the serum copper levels are directly proportional to the increase in the severity of OSMF [15].

In our study, the serum zinc levels increased from Group B. (1.000µg/ml) to Group C. (1.2228µg/ml), to Group A. (1.79µg/ml).

On studying the salivary zinc levels, it was found that the descending order was from Group A. (0.4472µg/ml), to Group B. (0.3821µg/ml), to Group C. (0.2740µg/ml).

Zinc is essential for the function/structure of several enzymes. At least 20 different zinc-containing proteins have been identified, which include anhydrase, alcohol dehyrogenase, leukocyte elastase and insulin [16]. There are conflicting reports in the literature on the decreased levels of serum zinc [8] and on the increased to the normal levels of zinc [17] in cancer patients.

In our study, increased levels of serum and salivary zinc were present in Group A. as compared to Group C., which could be due to the increased zinc transfer from the gutkha packets. If the chewing is on a regular basis, then the salivary zinc levels may remain raised for a longer time, thus allowing a greater absorption through the mucosa. These increased zinc levels can lead to an increased DNA synthesis and they could play a role in the carcinogenic potential of OSMF. While the decrease in the Zn levels in the diseases with benign characteristics appears to be less than that in the normal cancer cases [18], in malignancy, the Zn levels drop more [19]. OSMF, being a precancerous condition, may not classically show the dropping serum and salivary Zn levels .Its transition to malignancy and the changes in the Zn levels should then be evaluated.

The serum iron was found to be 0.6399 ± 0.3379µg/ml in Group A., 0.6030 ± 0.4561 µg/ml in Group B. and (0.836± 0.1998 µg/ml) in Group C. Thus, a steady decrease was observed from Group C to Group A to Group B.

The salivary iron levels were found to be 0.1037 ±0.1135µg/ml in Group A., 0.1064 ±0.996µg/ml in Group B. and 0.1589 ±0.0844µg/ml in Group C.

There was a decrease in the serum and the salivary iron levels in the Group A. patients as compared to those in the Group C patients; which was statistically significant. Iron is essential for the body as an oxygen-carrying pigment of the RBCs (haemoglobin), which accounts for 50% of the body iron. It also participates in the energy producing reactions (the cytochromes of the Kreb's cycle) in all the cells, it activates the energy producing oxidizing enzymes, it is necessary for DNA, RNA collagen and antibody synthesis, etc.

Most of the studies on the serum and salivary iron levels in OSMF and cancer have reported a decrease in the iron levels [20].

In our study, with respect to the iron levels, we hypothesized that the iron which was ingested by the patients via the gutkha products was utilized in the synthesis of collagen by the enzymes which were involved in the hydroxylation of proline and lysine. This could lead to a decrease in the proline levels and an increase in the hydroxyproline levels in the tissues of the oral submucous fibrosis individuals. This utilization may cause a decrease in the serum and the salivary levels in Group A. But since no such increased collagen activity was present in Group B, their iron levels could be increased or near normal.

The magnesium levels of serum and saliva were increased in the Groups A and B as compared to those in Group C. A similar increase was reported by other studies also [8,10]. Magnesium is known to play a vital role in the collagen biosynthesis. Hence, it may play some role in the aetiopathogenesis of OSMF. The increased levels of magnesium in our study group can be attributed to the ingestion of magnesium through the ingredients of gutkha.

CONCLUSION

In our study, the patients who had the habit of only gutkha chewing were included. It has been established that gutkha contains increased amounts of trace elements [3], which could contribute to the changes in the serum and the salivary levels as compared to those in the controls. These trace elements could play a key role in the aetiopathogenesis of OSMF. The role of copper has already been documented. Iron and magnesium play key roles in the biosynthesis of collagen along with copper; hence, more studies need to be conducted on these trace elements and on their roles in the pathogenesis of OSMF. Also, the malignant transformation rate of OSF was found to be in the range of 7–13%. Altered serum trace element levels are documented in the malignant cases and they are considered to be good biomarkers of malignancies. The serum copper and Zn levels and the Cu/Zn ratio in the OSMF patients can be considered as the markers for a susceptibility towards cancer.

The potentially strong points of this study are that the subjects with the gutkha chewing habits with and without OSMF were included, following stringent inclusion and exclusion criteria. Besides, atomic absorption spectrophotometry was used to analyze the elements, which is more accurate in the elemental analysis than the traditional calorimetry method.

It can be concluded from the present study, that the serum trace element levels could be used as potential prognostic and diagnostic markers in the OSMF patients.

REFERENCES

[1] Rajalalithap, Vali S, Molecular pathogenesis of Oral Submucous Fibrosis: A collagen metabolic disorder. *J Oral Pathol Med*. 2005;34:321-28.
[2] Babu S, Sesikaran B, Bhat RV. Oral Fibrosis among teenagers chewing tobacco, areca nut and pan masala. *Lancet*. 1996; 348: 692 .
[3] Ankolekar M, Karjodkar FR. Trace Elements and Microbiological Analysis. *JIAOMR*. January-March 2005; 17 (1): 11-17 .
[4] Pollack RL, Kravitz E. Nutrition in Oral Health and Disease; Philadelphia, Lea and Febiger. 1985.

[5] Thorling EB, Thorling K. The clinical usefulness of serum copper determination in Hodgkin's disease: A retrospective study of 241 patients from 1963-1973. *Cancer*. 1976;38: 225-31.
[6] Khanna S, Karjodkar F. Circulating Immune Complexes and trace elements (Copper, Iron and Selenium) as markers in oral precancer and cancer: a randomised, controlled clinical trial. *J IAOMR*. October 2006.
[7] Fisher GL, Byers VS Shifrine M, Levin AS. Copper and Zinc levels in serum from human patients with sarcomas. *Cancer*. 1976;37: 356-63.
[8] Vyas RK, Gupta AP, Gupta A, Aeron AK. Serum copper, zinc, magnesium and calcium levels in various human diseases. *Indian J. Medical Res*. 1982;76: 301-04.
[9] Oyama T, Kayamoto T, et al. A case-case study comparing the usefulness of serum trace elements (Cu, Zn and Se) and tumor markers (CEA, SCC and SLX) in non-small cell lung cancer patients. *Anticancer Research*, Jan-Feb 2003;23(1B):605-12.
[10] Rajendra R, Babu K, Mair KM. Serum levels of some trace and bulk elements in Oral submucous Fibrosis. *JDA*. 1992;63(6): 251-55 .
[11] Trivedi CR, Warnakulasuriya KAAS, Peters TJ, R Senkus, Karardy VK, Johnson NW. Raised tissue copper levels in OSMF. *J. Oral Pathol. Med*. 2000;29: 241-48.
[12] Trivedy C, Meghji S, Warnakulasuriya S, Johnson NW, Harris M. Copper stimulates human oral fibroblast in vitro – a role in the pathogenesis of Oral Submucous Fibrosis. *J. of Medicine*. 2001-11;30 (8): 465-70.
[13] Rajendran R, Kumari KR, Kumar AS. Liver ultrasound and faecal copper estimation in oral submucous fibrosis. *Ind J Dent Res*. 2003; 14: 13-21.
[14] Haque MF, Meghji S, Khitab U, Harris M . Oral submucous fibrosis patients have altered levels of cytokine production. *J Oral Pathol Med*. 2000;29: 123-28.
[15] Tadakamadla Jyothi, Santosh Kumar et al. Evaluation of serum copper and iron levels among oralsubmucous fibrosis patients. *Med Oral Patol Oral Cir Bucal*. 2011 Nov 1;16(7):e870-3.
[16] Gupta DS, Gupta M, Oswal RH. Estimate of major immunoglobulin profile in OSMF by radial immunodiffusion. *Int. J. Oral. Surg*. 1985;14: 533-37.
[17] Silverman S Jr, Thompson JS. Serum zinc and copper in oral/ oropharyngeal carcinoma: a study of seventy five patients. *Triple O* 1984;57(1): 34-36.
[18] Magalova T, Bella V, Brtkova A, et al. Copper, zinc and superoxide dismutase in precancerous, benign diseases and gastric, colorectal and breast cancer. *Neoplasma*. 1999;46(2): 100-04.
[19] Jayadeep A, Raveendran PK, Kannan S, et al. Serum levels of copper, zinc, iron and ceruplasmin in oral leukoplakia and squamous cell carcinoma. *Exp Clin Cancer Res*. 1997;16: 295-300.
[20] Rajendra R, Vasudevan DM, Vijayakumar T. Serum levels of iron and proteins in OSMF. *Ann. Dent. Winter* 1990; 49 (2): 23-25, 45.

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