ESTIMATION OF SERUM COPPER AND ZINC LEVELS IN PATIENTS WITH ORAL CANCER

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ABSTRACT

BACKGROUND

Oral cancer ranks number one among men and number three among women in India. As micronutrients deficiencies are common in India and have been related to oral and upper aerodigestive tract cancers, it is considered to be necessary as well to study the impact of nutrients. The current study reported is an attempt to suggest a positive role of micronutrients in prevention of cancer.

AIMS

The study was conducted to estimate the serum levels of copper and zinc in patients with oral cancer and to compare these values among patients with oral cancer and normal subjects.

STUDY DESIGN

The study group consisted of 30 oral cancer patients and 30 normal subjects.

MATERIALS AND METHOD

Serum levels of copper and zinc were estimated using semi-auto analyser in 30 oral cancer patients and 30 normal subjects.

STATISTICAL ANALYSIS

Data analysis was carried out using Statistical Package for Social Science (SPSS, V 10.5) package. One-Way Analysis of Variance were used to test the difference between groups and to find out which of the two groups means is significantly different post hoc test of Tukey test was used. In the above test, the "p" value of less than 0.05 was accepted as indicating statistical significance.

RESULTS

The mean serum levels of copper and Cu/Zn ratio were increased in oral cancer patients compared to normal subjects. The mean serum levels of zinc were decreased in oral cancer patients compared to normal subjects.

CONCLUSION

Serum levels of these trace elements maybe taken as prognostic markers of the disease progression in oral cancer patients.

KEYWORDS

Oral Cancer; Trace Elements; Serum Zinc; Serum Copper.

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INTRODUCTION

Oral cancer is one of the 10 most cancers in the world and shows a marked geographic difference in occurrence. On the basis of cancer registry data, it is estimated that annually 75,000 to 80,000 new cancer cases develop in India. Oral cancer ranks number one among men and number three among women in India. In the long incubation period between the initiation of carcinogenic habits and development of invasive oral cancer, well-defined oral precancerous lesions such as leucoplakia, submucous fibrosis, and erythroplakia occur.[1]

Tobacco chewing and smoking are strongly related to cancers mainly of upper aerodigestive tract. Several studies on diet and cancer links suggest that micronutrients, particularly antioxidants minerals are risk modifiers of cancer.[2] These include selenium, copper, zinc, manganese, and iron.[3] Many elements perform functions indispensable to maintenance of growth and reproduction. Inadequate levels of some elements may impair cellular and physiological functions. Trace elements have been critically examined in aetiology of various diseases, especially cancer.[4]

Several studies have shown that in cancer patients' serum copper levels are of considerable importance in assessing disease activity and prognosis. There are many reasons to assume that the presence of a malignant neoplasm may produce alterations in the micronutrient requirements of the patient. The rapid uncontrolled growth of malignant tissue produces a physiologic stress that may vary depending on the tumour.[5] Poor nutritional status may compromise immune competence and increase damage due to peroxidations.[2]

As micronutrients deficiencies are common in India and have been related to oral and upper aerodigestive tract cancers, it is considered to be necessary as well to study the impact of nutrients. Much more research is needed to characterise better markers of micronutrient status both in terms of metabolic effects and antioxidant effects so that at-risk patients can be identified and supplementation modified accordingly. The current study reported is an attempt to

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suggest a positive role of micronutrients in prevention of cancer.

MATERIALS AND METHODOLOGY

This study was conducted in the Department of Oral Medicine and Radiology, Government Dental College, Bangalore. 30 patients with oral cancer and 30 age and sex matched healthy controls formed the study group. Patient selection was based on following inclusion and exclusion criteria.

Inclusion Criteria

- Patients with a definitive diagnosis oral cancer both clinically and histopathologically were included in the study.

Exclusion Criteria

- Patients who have received treatment for oral cancer previously were excluded from the study.
- Patients with history of diabetes, hypertension, jaundice, liver or kidney disorders, or other systemic diseases and carcinoma elsewhere in the body were excluded from the study.

A detailed case history of the patient was taken and a thorough clinical examination was done and recorded on a standard proforma. Oral cancer patients were grouped clinically according to TNM staging given by American Joint Committee on Cancer[6] and histopathologically as per the Broder's classification.[7]

5 mL of venous blood was collected using aseptic measures from median cubital vein and sent to laboratories in sterile vials for estimation of serum copper and zinc levels. The blood was allowed to clot at room temperature for 1-2 hrs. and then serum was separated by centrifuging at 3000 rpm for 10 minutes. Serum copper and zinc levels were estimated using colorimetric method. This was followed by a histopathological confirmation of diagnosis by performing an incisional biopsy of the lesion.

SERUM ZINC ESTIMATION

Principle

Nitro-PAPS reacts with zinc in alkaline solution to form a purple coloured complex, the absorbance of which is measured at 575 nm. Interference from copper and iron are virtually eliminated by pH and chelating additives.

Components

Reagent A: 5 x 20 mL (liquid blue cap)

Composition

Borate buffer: 370 mM, pH 8.20
Dimethylglyoxime: 1.25 mM.
Salicylaldoxime: 12.5 mM.
Surfactants and preservatives.

Reagent B: 5 x 5 mL (liquid) red cap
Composizione: Nitro-PAPS 0.40 mM.

Standard: Zinc solution 200 µg/dL - 5 mL.

Reagent Preparation

Mix one vial of reagent B with one vial of reagent A.

Specimen: Serum.

Test procedure

Wavelength: 575 nm (allowed 570 + or - 582 nm).
Light path: 1 cm.
Temperature: 25, 30, or 37c.
Mix, incubate at 25, 30, or 37c for 5 minutes.
Read absorbances of standard (AS) and samples (Ax) against reagent blank.

Results Calculation

Serum zinc µg/dL = Ax/As x 200(standard value).

Expected Values: Serum zinc: 70 to 150 µg/dL.

SERUM COPPER ESTIMATION:

Methodology: Colorimetric method.

Principle

Copper as Cu (II) combines in acidic media with DiBr-PAESA to form a colour complex, the absorbance at which is measured at the interval of 570-590 nm. Sample blank can be avoided as well as serum deproteinisation.

Reagent A 2 x 25 mL (liquid) blue cap
Composition: Acetate buffer: 100 mM, pH 4.90,
Surfactants and preservatives.

Reagent B 2 x 25 mL (liquid) red cap
Composition: 3, 5 DiBr-PAESA: 10 mM.

Standard - 5 mL copper in diluted acid 200 µg/dI.

Reagents Preparation: Add a vial of reagent B to a vial of reagent A.

Specimen: Serum.

Manual Assay Procedure

	Reagent Blank	Standard/Sample
Standard/sample	_	100 µL
Reagent	1.5 mL	1.5 mL

Mix, incubate 10 minutes at 25, 30, or 37°C. Read absorbances of standard (As) and samples (Ax) at the allowed interval 570 - 590 nm against reagent blank.

Colour developed is stable at least 20 minutes away from strong light sources.

Calculations

Ax/As x 200 = µg/dL copper.

Expected Values

Men: 80 - 140 µg/dL, Women: 80 - 155 µg/dL.

Ethics

A formal ethical clearance to conduct this study was obtained by the Ethical Committee of the college. A formal informed written consent was obtained from all of them.

Statistics

The following methods of statistical analysis have been used in this study. Data analysis was carried out using Statistical

Package for Social Science (SPSS, V 10.5) package. One-Way Analysis of Variance were used to test the difference between groups and to find out which of the two groups means is significantly different post hoc test of Tukey test was used. In the above test the "p" value of less than 0.05 was accepted as indicating statistical significance.

RESULTS

Among 30 oral cancer patients, there were 20 males (66.7%) and 10 females (33.3%) patients. In patients with oral cancer, the mean age was found to be 55.70±12.65 years (mean±SD) with 30% in the age group of 50-59 years, 23.3% in the age group of 40-49 years, 20% in the age group of 60-69 years, 20% in the age group of 70-79 years, 3.3% of patients in the age group of 20-29 years and 3.3% of patients in the age group of 30-39 years.

21 (70%) patients with oral cancer had involvement of buccal region, soft palate was involved in 1 (3.3%), tongue in 1 (3.3%), lower alveolus in 5 (16.7%), and upper alveolus was involved in 2 (6.7%).

The clinical staging done for oral cancer as per the TNM staging given by American Joint Committee on cancer showed that 2 (6.7%) belonged to stage I, 3 (10%) belonged to stage II, 12 (40%) belonged to stage III and 13 (43.3%) belonged to stage IV. The histological grading of oral cancer patients as per Broder's classification showed that 13 (43.3%) were Grade I, 10 (33.3%) were of Grade II and III, 7 (23.3%) were of Grade IV.

The mean serum copper level in control group was 124.83±20.68 (mean±SD) µg/dL and 151.20±11.20 (mean±SD) µg/dL in patients with oral cancer. There was significant increase in the mean serum copper level. Oral cancer patients compared to mean serum copper level in control group. (Table 1).

The mean serum copper level showed an increasing trend through stage I to stage IV oral cancer patients. (Table 2). There was no significant difference in the mean serum copper level among the histological grades of oral cancer patients.

The present study showed that the mean serum zinc level in control group was 119.97±8.55 µg/dL (mean±SD) and in patients with oral cancer were 104.47±6.34 µg/dL (mean±SD) (Table 3). The mean serum zinc level was significantly decreased in patients with oral cancer compared with the mean serum zinc level of control group.

There was no statistically significant difference in the mean serum zinc level among the clinical stages and histological grades in oral cancer patients.

The mean serum Cu/Zn ratio in control group was 1.11±0.31 (mean±SD) and 1.45±0.15 (mean±SD) in patients with oral cancer. The mean serum Cu/Zn ratio was significantly increased in oral cancer patients when compared to control group. (Table 4).

The mean serum Cu/Zn ratio showed an increasing trend through clinical stage I to stage IV oral cancer patients. (Table 5).

There was no significant difference in the mean serum Cu/Zn ratio among the histological grades oral cancer patients.

	N	Mean	Std. Deviation	Minimum	Maximum	F 6.14	P Value <0.001
Control	30	124.83	20.68	70	154		
Oral Cancer	30	151.20	11.20	128	168		

Table 1: Mean Serum Copper Levels in Study Groups

	N	Mean	Std. Deviation	Minimum	Maximum	F	P value
Stage I	2	128.00	.00	128	128		
Stage II	3	135.33	5.51	129	139		
Stage III	12	148.75	3.74	143	154	47.44	<0.01
Stage IV	13	160.69	5.37	152	168		

Table 2: Comparison of Mean Serum Copper Levels Among the Clinical Stages in Oral Cancer

	N	Mean	Std. Deviation	Minimum	Maximum	F 7.98	P Value <0.001
Control	30	119.97	8.55	105	135		
Oral cancer	30	104.47	6.34	97	123		

Table 3: Mean Serum Zinc Levels in Study Groups

	N	Mean	Std. Deviation	Minimum	Maximum	F 5.43	P Value <0.01
Control	30	1.1114	.31566	.48	1.71		
Oral cancer	30	1.4538	.15264	1.13	1.70		

Table 4: Mean Serum Copper/Zinc Ratio Among Study Groups

	N	Mean	Std. Deviation	Minimum	Maximum	F	P value
Stage I	2	1.2367	.0084	1.23	1.24		
Stage II	3	1.2711	.1364	1.13	1.40		
Stage III	12	1.4284	.0996	1.21	1.56	8.242	.001
Stage IV	13	1.5527	.1291	1.32	1.70		

Table 5: Comparison of Mean Serum Copper/Zinc Ratio Among Clinical Stages in Oral Cancer

DISCUSSION

In the present study, the mean serum copper levels were increased significantly in patients with oral cancer when compared with that of normal subjects. Studies by J.N. Jha et al in 1985[8] and G.B. Toke in 1990[9] showed elevated levels of serum copper in patients with oral cancer compared to normal

subjects. It was stated by the authors that the rise in serum copper is possibly due to increase in serum cuproenzyme, ceruloplasmin consequent to decreased catabolism of this enzyme in cancer patients.[8] Fisher et al have suggested that a decrease in catabolism of ceruloplasmin as a possible cause of hypercupraemia seen in malignancy. Resialylation of ceruloplasmin could lead to decreased catabolism by liver and therefore hypercupraemia as binding to ceruloplasmin accounts for 96% of serum copper.[10] The involvement of copper ions in biological damage caused by superoxide, a radical found in all living tissues has been documented. According to this model, superoxide radical or other reducing agents such as ascorbate reduce the copper complexes to the cuprous state. In turn, these complexes react with hydrogen peroxidase to form hydroxyl radicals that damage proteins, RNA, and most important DNA. Repetitive formation of OH radicals at a specific location where the copper ions are found is probably the mechanism of this process. These radicals may cause double strand breaks in the cellular DNA that are not repairable by cellular mechanisms thus initiating a malignant process.[11]

The present study showed that mean serum zinc levels were decreased in patients with oral cancer compared to that of normal subjects. This finding was similar to that of the study by Iype Varghese et al in 1987.[12] It was stated that a decreased level of serum zinc associated with the carcinogenesis maybe due to increased utilisation of zinc by tumour tissues.

A decreased level of serum zinc in patients with oral cancer when compared with normal subjects was also observed by J. N. Jha et al in 1985[8] and G. B. Toke et al in 1990[9] in their studies. They stated that zinc, which is essential for DNA polymerase activity is particularly important in cell proliferation encountered in growing cell tumour. Perhaps the increased metabolic requirement of zinc by cancer cells results in an increased uptake from serum. Infection and tissue damage, which are often associated with oral carcinoma lead to liberation of leucocytes endogenous mediator from neutrophils, which in turn results in a decrease in serum zinc and concomitant uptake of zinc by the liver.[8]

It is also considered that hypozincaemia in malignant diseases is related to the decreased albumin fraction, which combines with about 60% of plasma zinc and serves as a transporter of the metal.[13]

Various immunologic abnormalities have been associated with zinc deficiencies in human beings. A mild deficiency of zinc is associated with decreased thymulin activity, reduced ratio of CD4+/CD8+ t-cells, and decreased production of interleukin-2. The fact that Il-2 plays a central role in maintenance of thymocytic and peripheral T helper cell population. The generation of antiviral and antitumour specific cytotoxic T-cells, up regulation of natural killer cell lytic activity implies that mild zinc deficiency could also lead to enhanced susceptibility to infection and malignancies by impairing production of this cytokine.[14]

The present study showed an elevation in mean serum Cu/Zn ratio in patients with oral cancer compared to normal subjects. Our results were similar to results of study by J.N. Jha et al in 1985[8] and G.B. Toke et al in 1990[9] who in their studies showed that there is an increase in level of serum Cu/Zn ratio in patients with oral cancer when compared to that of normal subjects. The value of serum Cu/Zn ratio increased significantly with the advancing stages of disease, which was also seen in our study groups. It was stated that serum copper is generally increased and serum zinc is generally decreased in cancer patients and also because serum zinc homeostasis is not well regulated as copper homeostasis, the ratio of serum Cu/Zn can be considered as a more reliable index of these two elements in patients with cancer of the digestive tract.

M. Abdulla et al in 1979 in their study observed there was a significant decrease in plasma zinc and increase in Cu/Zn ratio in plasma of patients with squamous cell carcinoma than in healthy controls. The plasma zinc level was estimated in the same patients after treatment. In patients responding to therapy, zinc and the copper/zinc ratio in the plasma and whole blood became normal. These results suggested a potential screening and predicting value of zinc and the copper/zinc ratio in plasma and whole blood in squamous cell carcinoma of the head and neck.[15]

In a study by S. Khanna et al in 2006 showed a gradual increase in serum copper levels from precancer to cancer group as compared to normal and suggested a positive role of copper ions in tissue damage.[16]

Study by Apeksha R.et al in 2010 showed increase in serum copper levels, decrease in serum zinc levels, and elevated levels of Cu/Zn ratio in patients with oral cancer similar to findings of our study. It was stated that elevated copper is comparable with that of other malignancies like lymphoma, lung cancer, and breast cancer. Association of elevated copper levels in oral cancer can be correlated to its role in tumour angiogenesis, which is responsible for tumour development and progression. Decreased levels of zinc could be because the malignant cells probably require more zinc, which is taken up from serum and hence there is a decreased serum level of zinc in patients with oral cancer. As there is negative interaction between copper and zinc, hence increases in copper levels will cause decrease in zinc level.[17]

Study by S. Khanna et al in 2013 demonstrates that there is an alteration in trace element profile in serum the OSMF and OSCC patients when compared to the control group. An attempt was made to assess these parameters as predictors for disease occurrence and progression. An analysis of the data indicates a direct relationship of serum Cu with the incidence of disease. The association of disease occurrence with Zn was not clear and less conclusive. It was concluded that Cu and Zn play an important role in oral carcinogenesis, but further research was warranted on larger population.[18]

Study done by Amith Kumar et al in 2014 showed the finding of low levels of zinc and copper associated with oral cancer patients. They also stated that copper/zinc ratio may serve as good indicator for the early detection of oral cancer and zinc deficiencies impair host protective mechanism designed to protect against DNA damage enhances susceptibility to DNA damaging agents and ultimately increase the risk of cancer.[19]

CONCLUSION

Our study showed change in serum levels of zinc, copper, and copper/zinc ratio in patients with oral cancer compared to that of normal subjects. On the basis of this study, it can be suggested that serum levels of these biochemicals may be used as prognostic markers in oral cancer patients and this biochemical assessment can be of value for proactive

intervention of high-risk groups. But to validate the above results, further studies on large sample size are required.

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