Estimation and Comparative Evaluation of Serum Iron, Copper, Zinc and Copper/Zinc Ratio in Oral Leukoplakia, Submucous Fibrosis and Squamous Cell Carcinoma

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Abstract

Higher incidence of oral cancer and precancer in Indian population necessitates in depth probing of various etiological and contributory factors for its early diagnosis and prognosis. In the present study serum iron, copper, zinc levels and Cu/Zn ratio were estimated in control and study groups [oral leukoplakia (OL), oral submucous fibrosis (OSMF), oral squamous cell carcinoma (OSCC)]. Estimation of these trace elements was done by colorimetric method using semi automated analyzer. Serum iron level and Cu/Zn ratio were significantly reduced while serum copper was increased significantly in OL, OSMF, and OSCC. The comparison of these trace elements between study groups was also found to be significant. Thus, assessment of Cu, Zn, Fe and Cu/Zn ratio can be used as auxiliary test to clinicopathological diagnosis and prognosis of oral precancer and cancer.

Keywords: OSMF, OL, OSCC, trace elements, iron, copper, zinc.

INTRODUCTION

Increasing incidence of oral cancer and precancer in Indian population necessitates in depth probing of various etiological and contributory factors for its early diagnosis and prognosis. At present confirmation of precancer and cancer is by histopathology. Thus, there is need to develop sensitive, specific and faster tests as an aid in the early diagnosis of the primary tumor and its recurrence or malignant transformation in premalignant states.

Recently, trace elements are receiving much attention in the detection of oral cancer and precancer as they are found to be significantly altered in head and neck, lung and breast carcinomas. Trace elements have an important role in carcinogenesis. Very few studies have been conducted to find out the role of different trace elements in oral precancer and cancer. Hence a comprehensive study has been carried out to estimate levels of serum iron, copper, zinc and Cu/Zn ratio in patients with OL, OSMF and OSCC in central India.

AIMS AND OBJECTIVES

This study has been carried out to estimate and compare serum iron, copper, zinc levels and Cu/Zn ratio amongst OL, OSMF and OSCC patients and to establish their relationship, if any.

MATERIALS AND METHOD

For the present study 120 patients were selected at random from Out Patient Department of Oral Medicine and Radiology, Government Dental College and Hospital, Nagpur. They were grouped as follows:

- Group I: 30 healthy individuals.
- Group II: 30 patients of oral leukoplakia.
- Group III: 30 patients of oral submucous fibrosis.
- Group IV: 30 patients of oral squamous cell carcinoma.

Patients from all the groups were thoroughly examined clinically. Each patient’s history was taken and recorded. OSMF patients were grouped clinically according to Khanna et al.¹ and oral cancer patients by AJCC tumor staging system with TNM parameter.² The diagnosis of the lesions (Group II, III and IV) were confirmed histopathologically. Serum iron, copper and zinc analysis were carried out at Department of Biochemistry, Government Medical College and Superspeciality Hospital, Nagpur.

Patients with systemic disorders, with history of drug intake containing iron, copper and zinc, with previous history of treatment for the same conditions, and pregnant, menstruating and patients on oral contraceptives were excluded from the study. For control group, in addition to above criteria, subjects with any habit of tobacco, betel nut and alcohol were excluded.

Under aseptic conditions 3 ml venous blood was withdrawn from each individual using sterile disposable syringe, transferred to plastic test tubes and kept standing for 30 minutes at room temperature. Then the serum was separated by centrifugation (R4A centrifuge machine) at 3000 rpm for 15 minutes. Hemolyzed samples were excluded. Precautions were taken to prevent the contamination of samples by other elements. Serum samples were stored at 2 to 8°C for 5 days and then analyzed.

Serum iron, copper and zinc were detected by colorimetric method using semiautomated analyzer, ‘Erba Chem-5 plus’ of...
Transasia Bio-Medicals Ltd. Laboratory and reagents used for this study were of “Crest Biosystems”, a division of Coral clinical systems. Procedure was performed as per manufacturer’s instructions.

Statistical analysis of the data was done using software ‘Intercooled Stata – Version 8.0’.

OBSERVATIONS AND RESULTS

The observations and results are summarized as follow:

The mean serum iron level in oral leukoplakia (104.03 ± 6.83 μg/dl), in OSMF (64.33 ± 8.20 μg/dl) and in OSCC (56.66 ± 5.78 μg/dl) reduced significantly (p < 0.01) compared to control (128.03 ± 5.56 μg/dl) (Graph 1).

The comparison of mean serum iron level between the study groups was highly significant (p < 0.01).

The mean serum copper in OL (114 ± 7.46 μg/dl), in OSMF (134.43 ± 6.43 μg/dl) and in OSCC (134.50 ± 4.83 μg/dl) increased significantly (p < 0.01) compared to control (104.9 ± 6.32 μg/dl). (Graph 2).

The difference between mean serum copper in OL and OSMF and between OL and OSCC was found statistically significant (p < 0.01).

No significant difference (p = 1.00) in mean serum copper level was found between OSMF and OSCC.

The mean serum zinc level in OSMF (78.90 ± 9.07 μg/dl) and in OSCC (59.03 ± 9.52 μg/dl) reduced significantly (p < 0.01) compared to control (96.30 ± 4.62 μg/dl) (Graph 3).

No significant (p = 0.2) difference in mean serum zinc in OL (91.96 ± 7.85 μg/dl) was observed compared to control.

The comparison of mean serum zinc level between OL, OSMF and OSCC was highly significant (p < 0.01).

The mean serum Cu/Zn ratio in OL (1.25 ± 0.14), in OSMF (1.72 ± 0.17) and in OSCC (2.33 ± 0.39) was increased significantly (p < 0.01) compared to control (1.09 ± 0.09) (Graph 4).

The comparison of mean serum Cu/Zn ratio in OL, OSMF and OSCC was statistically highly significant (p < 0.01).

The difference in mean serum iron level in control between males (129.8 ± 5.3 μg/dl) and females (126.2 ± 5.4 μg/dl) was nonsignificant. It was also nonsignificant in OL (males 104.3 ± 6.86 μg/dl and females 101 ± 7 μg/dl), in OSMF (males 65.04 ± 8.24 μg/dl and females 60.8 ± 7.82 μg/dl) and in OSCC (males 56.94 ± 6.06 μg/dl and females 56.18 ± 5.51 μg/dl).

The difference in mean serum copper level in control between males (103.9 ± 4.80 μg/dl) and females (105.8 ± 7.59 μg/dl) was nonsignificant. It was also nonsignificant in OL (males 113.9 ± 7.20 μg/dl and females 114.6 ± 11.5 μg/dl), in OSMF (males 134 ± 6.68 μg/dl and females 136.6 ± 5.02 μg/dl), in OSCC (males 133.4 ± 5.52 μg/dl and females 136.2 ± 2.72 μg/dl).

The difference in mean serum zinc level in control between males (97.4 ± 3.54 μg/dl) and females (95.2 ± 5.40 μg/dl) was nonsignificant. It was also nonsignificant in OL (males 92.11 ± 8.03 μg/dl and females 90.66 ± 7.37 μg/dl); in OSMF (males 79.72 ± 9.24 μg/dl and females 74.8 ± 7.72 μg/dl) and in OSCC (males 59.63 ± 9.63 μg/dl and females 58 ± 9.69 μg/dl).
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The difference in mean Cu/Zn ratio in control between males (1.06 ± 0.07 μg/dl) and females (1.11 ± 0.1 μg/dl) was nonsignificant. It was also nonsignificant in OL (males 1.24 ± 0.13 μg/dl and females 1.27 ± 0.22 μg/dl); in OSMF (males 1.69 ± 0.16 μg/dl and females (1.83 ± 0.17 μg/dl) and in OSCC (males 2.29 ± 0.38 μg/dl and females 2.41 ± 0.42 μg/dl).

The difference in mean age in OL (47.33 ± 14.46 years); in OSMF (26.46 ± 8.83 years) and in OSCC (53.06 ± 11.09 years) was statistically significant compared to control (37.53 ± 13.46), p values being p < 0.05, p < 0.01, p < 0.001 respectively.

Precancerous and cancerous lesions were seen more commonly in males than females, ratio being 9:1 in OL, 5:1 in OSMF and 1.7:1 in OSCC.

Tobacco (either chewing or smoking) was one of the commonest habit in OL and OSCC patients while areca nut (either alone or in forms of gutkha, kharr, betel quid) in OSMF, suggesting the cause and effect relationship.

DISCUSSION

Cancer is one of the leading causes of death in India. Many researchers suggested that it occurs due to the complex interactions of different etiologic factors. There is also a high rate of malignant transformation of premalignant states like oral leukoplakia and oral submucous fibrosis. Among premalignant conditions, OSMF is of special concern not only due to its crippling condition that it produces in the patient but as the entire oral mucosa acquires malignant potential.3


In the present study increased Cu/Zn ratio was increased in OL, OSMF and OSCC compared to control. This is in accordance with Abdulla et al11 (1979), Jha et al7 (1985), Toke GB et al8 (1990), Jayadeep et al9 (1997), Luquman et al10 (2004).

In the present study serum zinc level was significantly decreased in OSMF and OSCC and nonsignificantly decreased in OL compared to control.

Precancerous and cancerous lesions were seen more commonly in males than females, ratio being 9:1 in OL, 5:1 in OSMF and 1.7:1 in OSCC.

Recently much attention has been given towards detection of trace elements in oral cancer and precancerous states because of the encouraging results of the studies on head and neck carcinoma, lymphoma, lung carcinoma and breast carcinoma.

In present study serum iron was significantly decreased in OL, OSMF and OSCC compared to control. This finding is similar to Rajendran et al4 (1990) who found significant depression (p < 0.05) in serum iron level in oral leukoplakia and in OSMF compared to control. Vijaya Kumar T5 (1991) also found reduced levels of iron in OL, OSMF and OSCC.

In present study serum copper was significantly increased in OL, OSMF and OSCC compared to control. This is in accordance with Sashidhan et al6 (1983), Jha et al7 (1985), Toke GB et al8 (1990), Jayadeep et al9 (1997), Luquman et al10 (2004).

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Serum iron, copper and zinc levels are likely to change due to pregnancy, estrogen therapy, systemic illness like myocardial...
infarction, hypertension, diabetes mellitus, thyrotoxicosis, liver cirrhosis, alcohol consumption. As these possible factors except alcohol (as it is one of the etiologic agents in cancer development) were excluded from this study, altered levels can be correlated with the precancerous and cancerous states.

In the present study serum iron levels were decreased in oral leukoplakia, OSMF as well as in oral carcinoma patients. As all the subjects included were from same socioeconomic status, the lowered level of iron appears to be the effect of the disease process rather than its cause. It can be suggested that the lack of consumption of normal diet initiate anemia which further perpetuated by progression of disease.

The serum copper level was found increased in the present study in oral leukoplakia, OSMF and oral carcinoma. It was reported by Hrgovcic that elevated copper level is also associated with acute or chronic infections. Most of the patients of oral leukoplakia included in the present study had homogenous leukoplakia and were unaware of the lesion. They reported to the hospital with either periapical or periodontal infection, which may be responsible for the elevated level of serum copper in them.

Increased serum copper in OSMF can be correlated with findings of previous studies, i.e. the areca nut has high copper contents, OSMF patients have elevated tissue and salivary copper levels; and copper present in areca nut increases collagen production in oral fibroblasts by up regulating lysyl oxidase leading to cross linking of collagen and elastin. It holds true in the present study as all the patients of OSMF were areca nut chewers which is responsible for elevated serum copper levels in them.

Elevated level of copper in oral carcinoma is comparable with that of other malignancies like lymphoma, lung cancer and breast carcinoma. The increased level of copper is possibly due to an increase in cuproenzyme, ceruloplasmin consequent to its decreased catabolism. Alternatively, the elevated copper level may be due to chewing areca nut as there is increased incidence of oral carcinoma in areca nut chewers. Manousoso O, et al and Margiloth EJ et al also reported mutagenicity of copper in head and neck cancer. Association of elevated copper in oral carcinoma can be correlated with its role in tumor angiogenesis which is responsible for tumor development and progression.

The results of the present study show that copper plays an important role in OSMF and oral carcinoma. The progress of OSMF to oral cancer can be related to increased copper level and its mutagenicity.

In the present study the serum zinc levels in OSMF and oral cancer were significantly reduced. This could be because the malignant cells probably require more zinc which is taken up from the serum causing low levels of zinc in it. As there is negative interaction between copper and zinc, increase in copper level may cause subsequent reduction in zinc level as well.

Cu/Zn ratio was found to be significantly elevated in oral leukoplakia, OSMF and oral carcinoma in ascending order suggesting its correlation with disease progression.

CONCLUSION

Thus, from the present study, it is concluded that iron and zinc deficiency develop in patients with OL, OSMF and OSCC, and copper have an important causative role in OSMF and OSCC. The reduced levels of iron and zinc in descending order and increased copper level in ascending order from OL to OSMF and OSCC are helpful in assessing prognosis in these diseases.

Serum iron, copper and zinc levels are sensitive, but not specific, whereas serum Cu/Zn ratio is more reliable indicator in assessing progression of malignancy and the potential of transformation of premalignant state to malignancy. These can be used as an auxiliary test to clinicopathological diagnosis and/or in combination with other biochemical tests in the diagnosis and prognosis of oral precancer and cancer.

REFERENCES