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Role of Serum Trace Elements in Oral Precancer and Oral Cancer - a Biochemical Study

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Abstract Several studies in recent years have linked association between micronutrient levels and various forms of cancer. Copper and Zinc have been the most researched micronutrients. The aim of the study was to evaluate the levels of Copper, Zinc and iron in serum of patients with Oral leukoplakia, Oral Submucous Fibrosis and oral Squamous cell carcinoma. There was a highly significant increase in the level of serum copper in Oral submucous fibrosis patients when compared to controls (p=0.001). Serum copper levels were also elevated in oral leukoplakia and oral cancer patients (p=0.01). There was a significant decrease in the serum zinc levels in all three study groups when compared to controls (p=0.001). A highly significant reduction in serum Iron levels was noticed oral submucous fibrosis group. The copper to zinc ratio significantly increased in all the study groups when compared to controls. Results suggest that serum copper and zinc as well as the copper to zinc ratio could be used as biomarkers for oral precancer and cancer.

Keywords: *serum, copper, zinc, ratio, iron*

1. Introduction

India has one of the highest incidences of oral cancer in the world [1]. The development of cancer is a multistep process arising from pre-existing potentially malignant lesions. Leukoplakia is the most common precancer representing 85% of such lesions [2]. Alcohol, viruses, genetic mechanisms, candida and chronic irritation have modifying effects in the etiology of oral cancer [2]. Trace elements are regarded as versatile anti-cancer agents that regulate various biological mechanisms [3]. Many researchers have observed association between trace elements and cancer mortality [4]. Decrease in contents of Copper (Cu) and Zinc (Zn) in the blood of patients with head and neck cancer [5,6]. The ratio of copper to zinc is also believed to be a reliable biomarker in development and progression of carcinogenesis [7]. The present study was conducted to evaluate the levels of Cu Zn and Iron (Fe) in oral potentially malignant disorders and oral squamous cell carcinoma.

2. Materials and Methods

Two hundred patients (age range 20-60 years) reporting to the department of Oral medicine and Radiology were enrolled into the study over a period. Subjects with history of systemic illness, long term drug intake and concurrent malignancies were excluded from the study. Ethical clearance for the study was obtained from the institutional ethical committee. They were divided into four equal study groups with 50 subjects each. The first study group (OL) consisted of 50 patients (44 males, 6 females) of histopathologically confirmed cases of oral leukoplakia (4 homogenous leukoplakia, 46 speckeled leukoplakia). The second study group (OSMF) consisted of 50 patients with Oral Submucous fibrosis (47 males, 3 females). The third study group (OSSC) consisted of 50 histopathologically confirmed cases of oral squamous cell carcinoma (40 males, 10 females). The fourth study group (HC) consisted of 50 age and gender matched randomly selected healthy controls. Detailed history and clinical examination was done for each of the study subjects. After informed consent 5ml venous blood was collected from. The serum the centrifuged at 2500rpm for 10mins at 0-5℃. the supernatant were stored frozen at -20℃ until time of analysis. For the analysis of serum Cu and Zn levels a series of standard copper/ solutions were made raging from 1 to 10ppm. The unknown samples (study samples) were anylsed in comparison to the standardized Cu solutions using the GBC Avanta atom absorption spectrophotometer. Similar procedure was followed for estimation of serum Zn. Serum iron was measured by Bathophenanthroline method. 100 µL of the serum sample was taken in a clean microfuge tube and made upto 250 µL with the addition of deionised water. 500 µL of protein precipitating solution was added to the diluted sample. The mixture was then centrifuged at 2000rpm for 10 minutes. 500 µL of the supernatant was taken added to 500 µL of chromogen solution (25mg Bathophenanthroline sulfonate dissolved in 100mL of sodium acetate [2M]. The

optical density of the pink colour formed was read immediately (within 10 minutes) at 535nm against a blank treated in a similar method as the test wherein the sample was replaced with deionised water. The concentration of iron in the sample was deduced by plotting the optical densities of the test against the standard graph. The concentration that obtained was multiplied by the dilution factor (2.5). The data was statiscally analyzed by the ANOVA test using the SPSS version 17 software.

3. Results

The mean serum Copper levels in group HC, OSMF, OL and OSCC were 196.54 ± 3.76 µg/dL, 310.61 ± 4.22 µg/dL, 206.33 ± 2.57 µg/dL and 222.09 ±4.98 µg/dL respectively. There was a highly significant increase in the levels of Cu in the OSMF group when compared to controls. The Cu levels were also elevated in the OL and OSCC groups. However there was no statistically significant difference the groups OL and OSCC were intercompared (Figure 1).

The mean serum zinc levels in group HC, OSMF, OL and OSCC were 162.07± 2.91 µg/dL, 147.73 ± 2.86 µg/dL, 155.78 ± 2.03 µg/dL and 110.11± 2.83 µg/dL respectively. There was a highly significant reduction in the serum Zn levels in all three study groups OSMF, OL and OSCC when compared to HC. The Cu/Zn ratio was higher in all three study groups OSMF, OL and OSCC when compared to the HC (Figure 1).

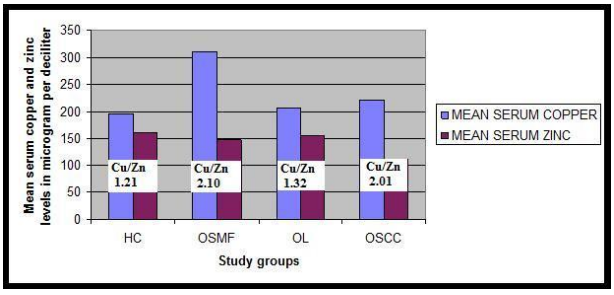

Figure 1. Graph showing men serum copper and zinc levels and the ratio of Cu/Zn

The mean serum iron levels in group HC, OSMF, OL and OSCC were 350.67 ± 2.65 µg/dL, 196.77 ± 2.34 µg/dL, 133.94 ± 2.32 µg/dL and 36.78±2.23 µg/dL respectively. The serum iron levels were least in the OSMF group (Figure 2).

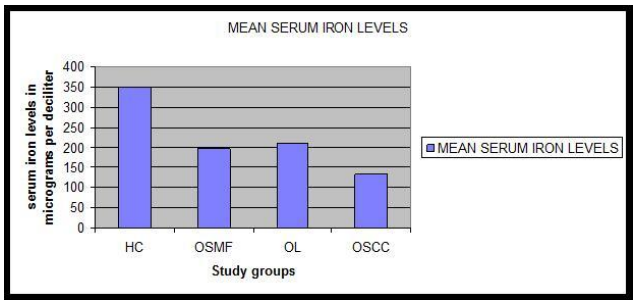

Figure 2. Graph showing mean serum iron levels

4. Discussion

Trace elements like copper and zinc have an role in the anticarcinogens defence system of the human body. Copper is involved in the cell metabolism, as a part of various enzymes tyrosinase, uricase and cytochrome oxidase, which are mainly concerned with oxidation reactions [8]. It was observed that mean serum copper levels were significantly higher in the sera of patients with oral premalignant and malignant lesions [9]. In our study there was an elevation of the serum copper in the oral leukoplakia and oral squamous cell carcinoma group. In one recent study it was observed that the levels of serum copper were elevated. In OSMF patients and gradually increased as the clinical staging OSMF progressed [10]. In our study the levels of serum copper was elevated significantly in OSMF patients.

Zinc acts a cofactor for Cu-Zn Superoxide Dismutase enzyme that is a part of the primary antioxidant system of all vertebrates [11]. Some studies have revealed lower zinc level in serum of patients with potentially premalignant disorders like in oral leukoplakia [12,13]. This might be because of the consumption of Zn in counter reacting to oxidants which are generated due to tobacco or high copper of areca quid metabolism [14]. In our study the serum levels of zinc was significantly reduced in all three study groups when compared to controls. Although there was no statistically significant difference between the serum Zn levels in oral leukoplakia and oral submucous fibrosis groups. Researchers also believe that zinc inhibits the invasive/migration activities in malignant prostate cells [15].

In our study we observed decreased salivary iron was observed in OSMF, OL and OSCC patients. Decreased iron levels in oral submucous fibrosis patients might be due to utilization of iron in collagen synthesis [16]. It has been stated that the decrease in iron content leads to decrease in epithelial vascularity which results in increased penetration of arecoline which leads to fibrosis [17]. Inadequate nutrition due to burning sensation and erosions in OSMF patients and increased tumor burden in OSCC patients are considered to be important factors for iron depletion [18]. Among the OSMF and OL study subjects 28 patients reported for yearly review. Only one speckled leukoplakia of the buccal mucosa case underwent malignant transformation to OSCC.

Future studies investigating the levels of Cu and Zn in precancerous and cancerous tissue and correlating them with serum changes would be useful in determining the role of these micronutrients in oral carcinogenesis. However the results of the present study reveal that Cu and Zn could be used as biological markers in oral carcinogenesis. Serum Cu to Zn ratio could also be used as a reliable biomarker.

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