

Serum copper and iron levels in oral squamous cell carcinoma patients: A south Indian study

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is one of the most common malignant tumors. Its incidence is rated as one among the highest in the world and the use of tobacco in various forms is increasingly associated with the cause of OSCC. Levels of serum copper and iron was estimated and compared among subjects with tobacco habits and healthy controls. OSCC patients were categorized according the tobacco habits. Serum estimation of copper and iron was done using atomic absorption spectrophotometry and spectrophotometry. In this study, the level of copper was significantly higher in OSCC patients with smoking habit than in non smokers. Significantly low levels of iron were found in OSCC patients with smoking habits. The present study revealed a progressive increase in level of copper with decreasing iron concentration in serum of OSCC patients. The study hypothesizes that this imbalance may be one of the major factors responsible for the progression of oral cancer.

Keywords: Oral squamous cell carcinoma, copper, iron, spectrophotometry, smokers.

INTRODUCTION

Oral cancer is the most common form of cancer in India. Its high risk in the Indian subcontinent is related to the popularity of tobacco products [1]. Oral cancer is the cancer found in the oral cavity; which is the sixth most common malignancy reported worldwide and one with highest mortality rate among all malignancies. Efforts directing at prevention, early detection and intensive therapy will go a long way in saving these patients [2]. Oral cancer has multifactorial etiology and is significantly associated with risk factors of the individual's lifestyle, particularly, use of tobacco, spicy food, alcohol and smoking.

It has been manifested that there is a dose dependent relationship between oral squamous cell carcinoma and tobacco consumption which is a common habit in Southeast Asia [3]. Malignant transformation rates as high as 7.6% have been reported from the Indian subcontinent over a 17 year period [4]. In order to explore for possible causes of malignancies on the one hand, and the need for a modality affording early diagnosis and follow up on the other, attention was paid to the role played by trace elements in malignancies [5].

Among many trace elements, copper and iron are required for the functioning of numerous enzymes and therefore it is reasonable to assume that variation in serum level of these biochemical markers may be associated with the pathogenesis of oral cancer and precancerous states [6]

Iron deficiency has long been known to have profound effects on the oral mucosa and also to have association with both oral and pharyngeal cancer (Paterson–Kelly syndrome), and with chronic candidiasis [7]. The findings of Joynson et al. [8] showed an impairment of cell mediated immunity in iron deficient patients. Deficiency as well as excess body iron, both may cause carcinogenesis. Some researchers emphasize that few habitual etiology have synergistic effect on iron to cause carcinogenesis [9]. Biochemical alterations of copper and iron concentrations in the serum of oral cancer patients can help not only in the early diagnosis, appropriate treatment but also as an indicator for prognosis [10].

Due to the higher incidence of oral cancer, the present study was undertaken to validate the level of copper and iron in OSCC cases and to correlate with the tobacco habits.

MATERIALS AND METHODS

A total of 40 patients (33 males and 7 females) in the age range of 55-75 years were selected for the study (Table 1), patients with histopathologically confirmed Oral Squamous cell Carcinoma only recruited into the study. According to TNM staging system of the UICC [11], out of 30 oral cancer patients studied, 19, 10 and 11 were of stages I, II and III respectively.

As controls, 40 (28 males and 12 females) age matched individuals with no systemic diseases were included in the study. Written informed consent was obtained from each case or control subjects before the data collection procedures were conducted. The work was carried out in accordance with the ethical standards laid down in 1964 Declaration of Helsinki.

Procedure of serum iron measurement:

Iron is released from transferrin through a decrease in the pH of serum. In an acidic medium transferrin bound iron dissociates into free ferric ions. Hydroxylamine hydrochloride reduces the ferric ions to ferrous ions which react with ferrozine to form a strongly colored purple complex with an absorption maximum near 560 nm.

One milliliter of reagent A (1.54 gm of Hydroxylamine hydrochloride in 0.306 gm of sodium acetate pH 4.5) was taken in each test tube placed for blank, standard and unknown sample. Then 200 μ l water in the blank, 20 μ l FeSO₄ solution and 150 μ l water in the standard and 200 μ l serum in the unknown sample tube was mixed. 40 μ l of reagent B (24 mg of ferrozine and 95.48 gm of hydroxylamine hydrochloride) was mixed in 6.2 ml water was then placed in each tube, mixed well and wait for 10-15 minutes. A turnery purple colored complex was formed. Then absorbance was measured in a spectrophotometer at 560 nm. Absorbance is proportional to iron concentration. Then the result was calculated to get the serum iron in μ g/dl.

Estimation of copper

Serum copper was measured by atomic absorption spectroscopy by using a method optimized by Centers for Disease Control on procedures from Instrumentation Laboratory, Inc.[12] and Perkin-Elmer Corp.[13].

Statistical analysis

All the results were expressed as the mean value \pm SD and statistical analysis was done by Students't' test. Data from the control subjects was compared with OSCC patients and a value of $P < 0.05$ was considered as statistically significant.

RESULTS

General characteristics of study group were shown in Table 1. Study subjects were composed of 33 male and 07 female individuals. In general, the mean age was 65.5 years. Subjects are classified into tobacco smokers (26) and non-smokers (14). Moreover, OSCC patients are categorized based on the tumor stages, Stage I (19), II (10) and III (11).

Table 1. General characteristics of the study subjects

Characteristics		Controls (n=40)	OSCC patients (n=40)
Age	Mean (yrs)	64.86±6.59	65.40±5.67
	Range (yrs)	55-75	55-75
Sex	Male	28 (70%)	33 (82.5%)
	Female	12 (30%)	07 (17.5%)
Smoking habit	Smokers	23 (57.5%)	26 (65%)
	Non Smokers	17 (56.6%)	14 (46.6%)
TNM staging	Stage I	-	19 (47.5%)
	Stage II	-	10 (25%)
	Stage III	-	11 (27.5%)

Table 2. Mean serum copper and iron concentrations in study group and control group

Category	Copper (µg/dl)	Iron (µg/dl)
Controls	112.09±2.56	129.21±1.10
OSCC Patients	131.72±2.07*	112.17±2.03*

*Significance in comparison to controls ($p < 0.05$).

The mean, standard deviation and P value of copper and iron levels in the oral cancer patients and controls were calculated and compared. There was a significantly increased level of mean serum copper and decreased level of iron was found in all groups of oral cancer patients as compared to the controls ($p < 0.05$). Based on the smoking habits, the values were compared among smokers and non smokers in the OSCC and control group (Table 3). The levels of copper is higher and level of iron was lower in tobacco smokers than non smokers ($p < 0.05$).

Table 3: Serum copper and iron levels in Oral Squamous Cell Carcinoma patients and controls according to their tobacco habits

Parameters	Controls (N=40)		OSCC Patients(N=40)	
	Smokers (n=23)	Non-smokers (n=17)	Smokers (n=26)	Non-smokers (n=14)
Copper (µg/dl)	113.12±0.95	111.10±0.82	123.1±6.35*	121.1±4.45
Iron (µg/dl)	126.12±0.87	128.16±1.11	109.11±2.09*	112.10±2.14

*Significance in comparison to controls ($p < 0.05$).

DISCUSSION

Life style and factors play an important role in the pathogenesis of oral cancer. Studies have shown an unequivocal correlation between tobacco chewing and smoking and oral squamous cell carcinoma [2]. Researches across the globe endeavored to elucidate possible relationship of trace elements with the risk of cancer in humans. Attempts of clinical correlation were also made by several clinical epidemiologists in this regard to establish a causal mechanism of malignancy inflicted by deficiency or excess of trace elements [8].

Biochemistry of iron, suggests that this metal may play an important role in carcinogenesis [14]. Meta analysis of studies conducted to uncover the relation of these trace elements with cancer risk failed to reach conclusive agreement. Our study revealed lower level of iron concentration in OSCC patients with smoking habits compared to non smokers.

The present study also showed that mean serum copper level was significantly high in subjects with smoking habits than in control group. In accordance with our study some researchers have indicated that smokers have significantly higher serum copper concentrations as compared to non-smokers [15,16]. Recently many investigators have sought to present significant information about the biochemical and pathobiochemical effects of trace elements in humans; such information on trace elements status may help to assess the risk of adverse outcome in OSCC patients. Whether trace elements supplementations become a target for prevention and therapy of OSCC will be the aim of future investigations.

CONCLUSION

It can be concluded from the present study that serum copper and iron levels could be used as a potential prognostic and diagnostic markers in OSCC patients. However, as there are controversial reports on the association of OSCC and these trace elements future studies are anticipated on a larger heterogeneous population to confirm the

hypothesis. Our results suggest that the high copper concentration and low iron concentration was found in smokers than non smokers among the oral cancer patients and correlates well with the disease progression. Consequently, these could be used as important parameters in the patients at risk for cancer and also draws attention as potential public health hazard.

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