

Efficacy of Antioxidant Vitamins and Trace Elements Level in the Prognosis of Oral Cancer

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ABSTRACT

Background & Objective: Imbalances between oxidant and antioxidant status have been implicated in the pathogenesis of oral cancer. Majority of oral cancer are headed by a well-recognized group of pre-malignant lesions. However, only a small fraction of those lesions undergo malignant transformation. Hence there is a great need to identify biological markers which will help in identifying lesion carrying high-risk. This study was aimed to determine serum levels of Beta carotene, Vitamin C, Ceruloplasmin, Zinc and Copper in patients with Precancer (Oral submucous fibrosis and Oral leukoplakia) & Oral cancer.

Methodology: The study consisted of 150 individuals with 50 healthy controls, 50 Pre-cancer patients (25 OSMF and 25 Leukoplakia) and 50 Oral cancer patients. Five ml of venous blood was drawn under aseptic precautions from these individuals and following tests by using Neeld and Pearson method for Beta carotene, Roe and Carl A. Kuether method for Vitamin C, Ravin H.A method for Ceruloplasmin, while Zinc and Copper by Kit method (Crest Biosystems) were performed and then the data was statistically analysed using student t-test.

Result & Interpretation: Significant (p value <0.05) reduced levels of Beta carotene, Vitamin C, Zinc and increased levels of Copper and Ceruloplasmin (<0.05) in patients with OSMF, Leukoplakia and Oral cancer was observed when compared with the controls.

Conclusion: Thus findings of the study suggest that the evaluation of these markers would be useful in assessing early malignant change, increasing accuracy of clinical diagnosis and also in assessing the spread and invasiveness of the cancer of oral cavity.

Key Words: Antioxidant Vitamins, Oral cancer, Trace elements, OSMF, Oral Leukoplakia.

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2395-6194.2015.00001.6

INTRODUCTION

According to the International classification of disease, cancers of the oral cavity include all the malignancies that are originating from the oral tissues such as lip, tongue, gum, floor of the mouth and unspecified parts of the mouth. More than 90% of oral cancers are mostly squamous cell carcinoma⁽¹⁾. The incidence and prevalence of oral cancer in India constitutes about 12% of all cancers in men and 8% in women⁽²⁾. The most unfortunate aspect of oral cancer is its high morbidity and mortality rates, despite the availability of varied treatment options. In India oral cancer is prevalent in most areas where tobacco related practises are observed. For development of oral cancer, tobacco is the single greatest risk factor. Oral Pre-cancers is defined as "Intermediate clinical state with increased cancer risk". Pre-cancers can be classified into two

broad groups: Pre-cancerous lesion and Precancerous condition.

Precancerous lesion is defined as "morphologically altered tissue in which oral cancer is more likely to occur than in its apparently normal counterpart" e.g. Leukoplakia and Precancerous condition is defined as a "generalized state associated with significantly increased risk of cancer" e.g. Oral submucous fibrosis. Ample evidence indicates that tobacco consumption, smoking, consumption of alcohol initiates the formation of reactive oxygen species and reactive nitrogen species. Either directly or through the activation of inflammatory cascade, ROS and RNS are proposed to play a key role both as initiators and promoters in carcinogenesis⁽³⁾. Additionally, there are considerable evidences that exist to prove the role of nutrients particularly Beta carotene, Ascorbic acid, Retinoids and alpha tocopherol etc. in prevention of cancer. Recently trace elements like Copper and Zinc are receiving much attention in detection of oral cancer and precancer as their levels were found to be significantly altered in head and neck cancers.

The extracts of areca nut acts as a potent stimulator for collagen synthesis in human fibroblasts culture leading to excessive accumulation of collagen, leading too fibrosis⁽⁴⁾. High levels of copper in areca nuts, a major etiological factor in oral submucous fibrosis plays an

initiating role in stimulation of fibrogenesis by up-regulation of lysyl oxidase and thereby causing inhibition of degradation of collagen causing its accumulation. The high serum copper levels may also lead to generate high levels of free radicals by metal catalysed Haber- Weiss reaction and this can be one of the reasons for the carcinogenesis in tobacco and areca nut users⁽⁵⁾.

Antioxidants are enzymes or other organic molecules that can counteract the damaging effects of reactive oxygen species in tissues⁽⁶⁾. Antioxidant nutrients such as Vitamin C, Beta -carotene, Zinc and Selenium are regularly found to reduce the risk of oral cancer as well as pre-cancers.

Thus, the present study is undertaken to comprehend the efficacy of these antioxidant vitamins and minerals level in the early diagnosis and prognosis of oral cancer.

MATERIAL AND METHOD

This study was conducted in Department of Oral Medicine and Radiology, People's College of Dental Sciences & Research Centre Bhopal. The study consisted of total of 150 individuals. These individuals were divided into the following study groups:

Group A-50 people with Oral Precancer (Leukoplakia/OSMF).

Group B-50 people with Oral Squamous cell carcinoma.

Group C-50 healthy individuals (controls) with no deleterious habits (non-smoker, non-tobacco / Areca nut chewer & non Alcoholic) & without any other oral lesions.

Beta carotene was estimated by Neeld and Pearson method. Vitamin C was estimated by 2-4 Dinitrophenylhydrazine (Roe and Carl A. Kuether) method. Ceruloplasmin was estimated by p-Phenylenediamine (Ravin H.A) method. Copper and zinc was estimated colorimetrically.

The results were expressed as mean \pm SD values. The mean of the patients and controls were compared using the Student's t test.

RESULTS

Table 1 shows the levels of serum B carotene, vitamin C, Ceruloplasmin, Zinc and Copper level in healthy control subjects and in oral precancer and oral squamous cell carcinoma patients. Table 2 shows the level of serum B carotene, vitamin C, Ceruloplasmin, Zinc and Copper level in oral precancer and oral squamous cell carcinoma patients. Table 3 shows the level of antioxidant vitamins and minerals level in different grades of oral cancer. Except for Ceruloplasmin the result of all the other parameters was found to be statistically significant when compared in different stages of oral cancer.

Table 1: Levels of Serum Beta Carotene, Vitamin C, Ceruloplasmin, Zinc and Copper in Study and Control Group

Parameters	GROUP A	GROUP B	GROUP C
	Mean \pm SD	Mean \pm SD	Mean \pm SD
β Carotene (μ g/L)	135.49 \pm 39.31	93.54 \pm 16.45	636.42 \pm 46.43
Vitamin-C (mg/dl)	0.50 \pm 0.26	0.376 \pm 0.26	1.09 \pm 0.172
Ceruloplasmin (U/L)	97.60 \pm 16.92	113.84 \pm 17.56	49.06 \pm 6.83
Zinc (μ g/dl)	64.90 \pm 15.20	44.44 \pm 6.48	112.48 \pm 10.31
Copper (μ g/dl)	147.54 \pm 19.26	166.23 \pm 12.37	100.97 \pm 21.03

Table 2: Levels of Serum Beta Carotene, Vitamin C, Ceruloplasmin, Zinc and Copper Osmf & Leukoplakia

Parameters	CONTROL Mean \pm SD	OSMF Mean \pm SD	Leukoplakia Mean \pm SD	P value	Remark
β Carotene (μ g/L)	636.42 \pm 46.43	148.27 \pm 37.80	124.26 \pm 37.76	0.0236	Statistically Significant
Vitamin-C (mg/dl)	1.09 \pm 0.172	0.42 \pm 0.238	0.59 \pm 0.26	0.0322	Statistically Significant
Ceruloplasmin (U/L)	49.06 \pm 6.83	102.16 \pm 16.33	93.04 \pm 16.56	0.0558	Not Quite Statistically Significant
Zinc (μ g/dl)	112.48 \pm 10.31	70.31 \pm 13.80	59.48 \pm 14.84	0.0102	Statistically Significant
Copper (μ g/dl)	100.97 \pm 21.03	157.32 \pm 15.92	137.52 \pm 17.45	0.0001	Extremely Statistically Significant

Table 3: Levels of Serum Beta Carotene, Vitamin C, Ceruloplasmin, Zinc and Copper in Different Grades of Oral Cancer

Table for Different Grades of Oral Cancer					
Parameters	Well Differentiated Mean \pm SD	Moderately Differentiated Mean \pm SD	Poorly Differentiated Mean \pm SD	P value	Remark
β Carotene (μ g/L)	105.05 \pm 9.336	93.62 \pm 10.84	73.74 \pm 18.55	0.0005	Extremely Statistically Significant
Vitamin-C (mg/dl)	0.568 \pm 0.21	0.348 \pm 0.226	0.112 \pm 0.079	0.0035	Statistically Significant
Ceruloplasmin (U/L)	107.08 \pm 16.76	111.82 \pm 15.18	129.94 \pm 15.19	0.3556	Not Quite Statistically Significant
Zinc (μ g/dl)	45.37 \pm 7.78	45.61 \pm 4.819	40.17 \pm 6.21	0.0104	Statistically Significant
Copper (μ g/dl)	159.58 \pm 11.61	165.88 \pm 7.369	178.30 \pm 14.66	0.0001	Extremely Statistically Significant

DISCUSSION

Oral cancer is the sixth most common cancer worldwide; its development is a multistep process arising from pre-existing potentially malignant lesions and conditions. Increasing incidence of oral cancer and pre-cancer in Indian population necessitates in depth probing of various etiological and contributory factors for its early diagnosis and prognosis. The tobacco use is one of the main causes for oral cancer along with alcohol; additionally, there are considerable evidences that exist to prove the role of nutrients in prevention of cancer.

In the present study the serum Beta carotene, Vitamin C and Zinc were decreased and serum Copper and Ceruloplasmin were increased in pre-cancer group as compared to controls which is in accordance with the studies conducted by Varghese Iype et al⁽⁷⁾ for Zinc, Khanna S Sunnali⁽⁸⁾ for Copper and Balpande R Apeksha et al⁽⁹⁾ for Copper and Zinc, and Rai B et al⁽¹⁰⁾ for serum and salivary Vitamin C in pre-cancer group.

In the present study serum Beta carotene level was significantly decreased in OSMF when compared to controls. Similar findings were observed in the other studies^(11,12,13). In Leukoplakia also serum Beta carotene levels were decreased and these values were at par with study conducted by Ramaswamy G et al⁽¹⁴⁾. The reason attributed for these decreased levels in both the condition/ lesion could be due to utilization of antioxidants by affected tissues or in combating the excessive oxidative stress in circulation⁽¹³⁾. Serum Beta carotene level was also significantly decreased in Oral cancer when compared to controls. Similar findings were observed in the other studies^(15,16,17,18,19). The reason for this decrease in values could be due to increased compensatory antioxidant defence in serum due to its utilization by affected tissues⁽¹⁶⁾.

In this study serum Vitamin C levels in OSMF was significantly decreased as compared to control group which is in accordance with study conducted by Wahi P. N et al⁽²⁰⁾, where the frequency of Vitamin C deficiency was significantly higher in cases of OSMF

than in control cases. The possible mechanism for this decrease could be that in OSMF patients, there is an increase in the production of highly cross linked insoluble collagen type I, loss of more soluble pro-collagen type III and collagen type VI. The cross linking of collagen due to the up-regulation of lysyl oxidase, plays a crucial role in the development and progression of the condition from stage I to stage II. Vitamin C levels decrease perhaps because of its utilization in collagen synthesis⁽²¹⁾. Similarly in Leukoplakia Vitamin C levels were significantly reduced which are in accordance with the other studies^(14,10) where they concluded that poor nutritional status and oxidative stress in pre-cancer lesions and conditions like OSMF are responsible for decrease in serum and salivary Vitamin C⁽¹⁰⁾. Vitamin C levels in Oral cancer was significantly decreased as compared to control group which is in accordance with study conducted by Kapil U et al⁽¹⁹⁾. The possible reason for this decrease in Vitamin C levels could be due to its action, as it scavenges free radicals and reactive oxygen molecules, which are produced during metabolic pathways of detoxification. It also reduces the development of nitrosamines from nitrates which act as carcinogens thus connection between the incidence of cancer and a dietary deficiency of Vitamin C or low blood levels in the body could be postulated⁽²²⁾.

In the present study serum ceruloplasmin levels in OSMF was significantly increased when compared to the controls. These observations are in agreement with observations of Renuka J Bathi et al⁽²³⁾. The increased values of enzymes in OSMF indicate increased antioxidant activity in this group, in response to the higher oxidative damage⁽²³⁾. Serum Ceruloplasmin levels were also increased in Leukoplakia which is at par with the other studies^(24,25). Between OSMF and Leukoplakia in the Pre-cancer group the serum ceruloplasmin levels were more raised in OSMF compared to Leukoplakia which was statistically significant. Ceruloplasmin is a Copper carrying enzyme, as in OSMF due to areca nut consumption

there is high Copper content in serum thus it can be postulated that Ceruloplasmin and Copper are interrelated. Also it is an effective antioxidant and organisms might respond by raising the antioxidant efficiency of plasma, i.e. by elevating the enzyme levels⁽²⁶⁾. In the present study serum Ceruloplasmin levels in oral cancer was significantly increased when compared to the controls these observations are in agreement with other studies^(24,16,25). The possible reason for this could be that Ceruloplasmin being an effective antioxidant protein and it is one of the acute phase reactants, whose concentration in plasma rises after tissue injury. Most acute phase proteins can be thought of as protecting the organism as a whole, from the possible ill effects caused by their release of free radical oxidation products. This suggests that the organism might respond to ongoing free radical damage by raising the antioxidant efficiency of plasma, by elevated Ceruloplasmin levels⁽²³⁾.

In the present study mean serum Copper level was significantly increased in OSMF compared to control this is in accordance with other studies^(27,8,28,21,9,29). Similarly, in the present study serum Copper levels were increased in Leukoplakia which is at par with the other studies^(24,27,8,9). Whereas comparison between OSMF and Leukoplakia in the Precancer group the serum Copper levels were more raised in OSMF compared to Leukoplakia which was statistically significant. The possible cause for the increase in serum Copper in OSMF can be correlated with previous studies, i.e. areca nut has high Copper contents, and OSMF patients have elevated tissue and salivary Copper levels; and Copper present in areca nut increases collagen production in oral fibroblasts by up-regulation of lysyl oxidase activity leading to cross linking of collagen and elastin⁽²¹⁾. In the present study serum Copper level is increased in oral cancer group compared to controls which is in accordance with the other studies^(24,8,30,31,32,33,34,35,9). Elevated level of Copper in oral carcinoma is comparable with that of other malignancies like lymphoma, lung cancer and breast carcinoma^(31,36). The increased level of Copper is possibly due to increase in cupro-enzyme, 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⁽⁹⁾. Manousoso O and Margiloth EJ et al⁽²¹⁾ 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⁽⁹⁾.

In the present study mean serum Zinc was significantly decreased in OSMF compared to control similar findings were observed in other studies^(28,7,9). Similarly, in the present study serum Zinc levels was decreased in Leukoplakia which is in concurrence with the other studies^(24,27,9). The reason attributed for the decrease of

serum Zinc levels in OSMF could be because there is negative interaction between Copper and Zinc, increase in Copper level may cause subsequent reduction in Zinc level as well the role of Copper cannot be segregated from that of Zinc because of the well elucidated biochemical relatedness^(27,9). Another study conducted by Kapil U et al⁽¹⁹⁾ also concluded Zinc levels to be low in laryngeal cancer patients when compared to controls which is similar to the present study. The reason attributed for this decrease in the values could be because the malignant cells probably require more zinc which is taken up from the serum causing low levels of Zinc in it⁽⁹⁾.

In the present study it was also found values of Beta carotene, Vitamin C and Zinc were decreasing and Copper and Ceruloplasmin were increasing gradually as the oral cancer progressed from well differentiated to poorly differentiated carcinoma which is in accordance with the study conducted by Lian SL et al⁽³²⁾ for Copper and Zinc stating that patients with more advanced stages of the disease had more elevated Copper and Zinc ratios. Another study conducted by S. Manoharan et al⁽³⁷⁾ assessed the levels of antioxidants with various clinical stages in oral cancer patients and they found gradual reduction in the levels from stage II to stage IV thus concluding that altered lipid peroxidation in plasma of oral cancer patients may be related to their compensatory changes in the antioxidant defense system⁽³⁷⁾.

CONCLUSION

From the present study it is evident that by estimation of serum antioxidants and trace elements in circulation of OSMF, Leukoplakia and Oral squamous cell carcinoma patients, one can assess the degree of oxidative damage of the disease. This study substantiates the concept that these antioxidants and trace elements be one of the important factors involved in causation of Oral Pre-cancer and Cancer in association with other contributing factors. Reduced levels of Beta carotene, Vitamin C, Zinc and increased levels of Copper and Ceruloplasmin in patients with OSMF, Leukoplakia and Oral cancer are helpful in assessing prognosis of these diseases.

Further correcting the underlying deficiencies of these antioxidants the treatment plan can be improved this in turn may be helpful for successful management of these diseases there by arresting them in early stage and avoiding possible consequences. However further elaborate studies with larger sample size along with follow up are needed to ascertain the actual role of these parameters in initiation and promotion of carcinogenesis. This will help the mankind in prevention from oral cancer.

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