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RESEARCH ARTICLE

ROLE OF SALIVARY VITAMIN C IN ORAL MALIGNANT AND POTENTIALLY MALIGNANT DISORDERS

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ABSTRACT

Oral cancer is reported as the sixth most frequent malignant neoplasm in the world. Among all malignancies 25% are found in the oral cavity. Early diagnosis of malignant and potentially malignant disorders may help in reducing mortality. Clinical efforts in cancer therapeutics were concentrated mainly on invasive cancer. Such an approach has limited effect. A refocus on carcinogenesis is important with prime aim for cancer prevention. Plasma concentrations of ascorbate have been shown to be inversely associated with risk for developing cancer. The objective of study was to estimate and correlate the salivary levels of vitamin C in oral malignant and potentially malignant disorders. 20 subjects with oral cancer and 20 subjects with potentially malignant disorders were selected and salivary levels of vitamin C were compared with controls. 1ml of whole saliva was collected from each subject. The samples were collected in micro centrifuging tube and were frozen at less than minus 20^0C. The samples were analyzed in a spectrophotometer for vitamin C levels. It is observed that salivary levels of vitamin C were very much reduced in oral malignant and potentially malignant disorders when compared to control ($p<0.001$). In the control group salivary vitamin C levels were maintained in all the age groups. Low levels of vitamin C causes increased cell damage. There is an inverse association of vitamin C and development oral cancer. Thus this study suggests that Vitamin C may have a strong association in inhibiting initiation and promotion of oral cancer.

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INTRODUCTION

Oral cancer is reported as the sixth most frequent malignant neoplasm in the world. Among all malignancies 25% are found in the oral cavity. Even though tobacco is the major cause of oral cancer, alcohol synergistically acts causing malignant changes. Tobacco, either smoked or smokeless form is responsible for 75% of oral cancer (Harinder Garewal, 1995). Most cancers of oral cavity are squamous cell carcinoma. Early diagnosis of malignant and potentially malignant disorders may help in reducing mortality. In a World Health Organization (WHO) Workshop, held in 2005, the terminology of oral lesions predisposing to malignant transformation was discussed.

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The term 'potentially malignant' was preferred above 'premalignant' or 'precancerous'. It was recommended to use the term 'potentially malignant disorders' to avoid the distinction between potentially malignant lesions and potentially malignant conditions. Oral Leukoplakia, Erythroplakia, Lichen Planus and Oral Submucous Fibrosis are commonly considered under potentially malignant disorders (Isaac van der Waal, 2008). Carcinogenesis is a multi step process leading to invasive cancer as its final stage. Carcinogenesis involves several sequential stages: Initiation, promotion and progression (Vishwa N Singh, 1991). Clinical efforts in cancer therapeutics were concentrated mainly on invasive cancer. Such an approach has limited effect, particularly when aim is prevention. As importance was given on final stage of cancer, it resulted in little effect on cancer control and eradication. A refocus on carcinogenesis is important with prime aim for cancer prevention. Tumor promotion is a slow process in humans. The latency period of many malignant neoplasms are 10-30 years.

Promotion is generally reversible. Since it takes long time, it is ideal for intervention with chemo preventive agents. A potentially malignant disorder can be either made to regress, or the rate of conversion to malignancy can be slowed down. Vitamins are essential nutrients that are required for various biochemical and physiological processes in the body. It is known that most vitamins cannot be synthesized in the body. Hence dietary supplementation is necessary. Vitamin C is a water soluble vitamin. It was first isolated in 1923 by Hungarian biochemist and Nobel laureate Szent-Gyorgyi and synthesized by Howarth and Hirst (Shailja Chambial *et al*., 2013). Since the discovery of vitamin C, the number of its known biological functions are expanding. The body requires vitamin C for normal physiological functions. It increases the absorption of iron in the gut by reducing ferric to ferrous state. It is a strong antioxidant. It protects the body from various toxic effects of free radicals. The recommended dietary intake for vitamin C is 75-125 mg daily. Ascorbate is essential for the full function of an array of enzymes. Adequate intake of vitamin C will optimize the metabolism and prevent cancer and other degenerative diseases. The concentration of ascorbate in plasma of healthy humans is about 40-80muM. These levels of ascorbates functions as an endogenous antioxidant. It acts as co-antioxidant with vitamin E to protect low density- lipoprotein (LDL) from oxidative damage induced by aqueous peroxyl radicals.

The chemo preventive role of vitamin C was 1st proposed by Cameron *et al* in 1949. Vitamin C is also known to inhibit N-Nitroso compounds which are known to induce carcinogenesis (Soitirious, 1987). Various studies showed that consumption of vitamin C rich foods reduces the risk of developing cancer. Plasma concentrations of ascorbate have been shown to be inversely associated with risk for developing cancer (Juan *et al*., 2012). The study of potentially malignant disorders has shown to be associated with development of cancer at subsequent stages. This gives an opportunity for identification and evaluation of efficacy of cancer inhibitory agents. Currently, epidemiological and biochemical studies of potentially malignant disorders are limited in number. This study is conducted to analyze the salivary vitamin C in oral malignant and potentially malignant lesions and evaluate the possible role of vitamin C in chemoprevention.

MATERIALS AND METHODS

Study was conducted among patients who visited our out patients department of Oral Medicine and Radiology, Vinayaka Missions Sankarachariyar Dental College, Salem. The control group was selected among the patients who came for routine examination and oral prophylaxis. Patients were explained about the need for study and a written informed consent was obtained from the patients included in both the study and control group. 20 patients with oral cancer between the age group of 40 and 80 years of age and 20 patients with potentially malignant disorders between the age group of 20 and 60 years of age were included in this study. The clinical diagnosis was histologically confirmed. Patients with histologically proven potentially malignant and malignant lesions were included in the study. Patients who were having vitamin deficiency and under vitamin therapy were excluded from the study 20 healthy. Individuals were selected as control in the age group of 20 and 65 years of age.Patients with vitamin deficiency and under vitamin therapy were excluded from the study. A thorough examination of the patient and detailed case history was taken. Individually informed consent was obtained from all subjects in the study. All selected patients were explained about the need of the study. Potential benefits of investigations using saliva were explained to the subjects. 1ml of unstimulated whole saliva was collected in a micro centrifuging tube (MCT). Saliva was collected at least one hour after food in MCT and kept over ice before preserving at -20 degree Celsius for analysis. All the tests were done with utmost care and aseptic conditions with use of sterile diagnostic instruments and well sterilized laboratory apparatus. Cross infections between doctor and patients was prevented by the use of disposable sterile gloves, mouth mask and head cap. The samples were centrifuged at 14000 rpm for 10 minutes and estimated for salivary vitamin C using the principle of colorimetry in a spectrophotometer.

RESULTS

A total of 60 patients were included in this study. Analysis of variance (ANOVA) test was used to analyze the statistical significance of salivary vitamin C levels in oral cancer and potentially malignant disorders with the control. 'P' value < 0.01 was considered to be statistically highly significant at 1% level, 'P' value between 0.011-0.05 was considered to be statistically significant at 5% level and the 'P' value of greater than 0.05 was considered to be statistically not significant at 5%level. The mean salivary vitamin C levels in control group were 231.45µmol/l. In oral cancer and potentially malignant disorders, the mean salivary vitamin C levels were 35.15 and 68.82µmol/l. The results were statistically highly significant ($p < .001$) when vitamin C levels in oral cancer and potentially malignant disorders were compared with control (Table.1)

In the age group up to 30 years, the mean salivary vitamin C levels in control group were 240.03µmol/l and in potentially malignant disorders the vitamin C levels were 150.24µmol/l. No oral cancer patients were present in this age group. In the age group of 31-40 years, the main salivary vitamin C levels in control group were 301.08µmol/l and in oral cancer and potentially malignant disorders it was 68.21µmol/l and 31.97µmol/l respectively. In 41-50 years age group the salivary vitamin C levels were 187.55µmol/l in control group. In oral cancer the vitamin C levels were 15.88µmol/l and in potentially malignant disorder the mean salivary vitamin C levels were 41.05µmol/l. In this age group the difference in salivary vitamin C levels were highly significant ($p<.001$) between control group and others. Age group of 51-60 years showed salivary vitamin C levels of 189.60µmol/l in control group and 43.49 µmol/l and 73.97µmol/l in oral cancer and potentially malignant disorders respectively. Above 60 years of age the vitamin C levels were 182.22µmol/l in control group.12.44µmol/l in oral cancer and 119.94µmol/l in potentially malignant disorders (Table.2).

DISCUSSION

This study was done to evaluate and compare the salivary vitamin C levels in malignant and potentially malignant disorders in oral cavity. Three groups with 20 subjects were considered in this study. Group1 with histologically proven oral cancer. Group 2 with histologically proven potentially malignant disorders and group 3 –control with no mucosal changes. After obtaining an informed consent from each patient included in the study, a detailed clinical examination was done and 1ml of whole saliva was collected in a MCT.

The samples were stored in a plasma freezer at a temperature less than -20 degree Celsius. The samples were transported and centrifuged for the analysis of salivary vitamin C in a spectrophotometer. The salivary levels of vitamin C were compared in each group.

Table 1. Mean age and salivary vitamin C levels

	Age				Group Total		t	P
	Male		Female		Mean	SD		
	Mean	SD	Mean	SD				
Control	36.64	11.37	46.33	13.13	41.00	12.85	1.77	0.094
Oral Cancer	54.20	13.72	59.00	6.52	55.40	12.34	0.74	0.466
Potentially malignant Disorders	48.38	11.16	42.50	15.59	47.20	11.94	0.87	0.393
ANOVA	6.67		2.41					
P	0.003**		0.124					

Table 2. Salivary vitamin C levels in different age group

		N	Mean	SD	ANOVA	P
Age	Control	20	41.00	12.85	6.81	0.002**
	Oral Cancer	20	55.40	12.34		
	Potentially malignant Disorders	20	47.20	11.94		
	Total	60	47.87	13.55		
Salivary Vitamin C	Control	20	231.45	109.76	34.24	< 0.001**
	Oral Cancer	20	35.15	50.43		
	Potentially malignant Disorders	20	68.82	68.70		
	Total	60	111.81	117.00		

The result suggested that the salivary levels of vitamin C in malignant and potentially malignant disorders were highly reduced when compared to the control group. The vitamin C levels in oral cancer were reduced when compared to the potentially malignant disorders but there was no statistical significance. In this study the prevalence of malignant and potentially malignant disorders were high in males when compared to females. There were no oral cancer patients below 40 years of age and the average age was 54 years. This is in accord with the statement of Greenberg and Glick in 2003 that oral cancer is a disease of older age group and 95% of cases occur in people above 40 years with an average of 60 years of age (Greenberg, 2003). This study suggests that there is a strong inverse association between vitamin C levels and oral cancer which is in favor of statement by Alexander J Michels in 2013 that many cohort study have observed the inverse association between Vitamin C intake or plasma levels and the incidence of cancer (Alexander, 2013). Anitra C Carr in 1999 also concluded that a consistent inverse association exists between vitamin C intake and oral cancer (Anitra, 1999). When compared with control group, the salivary vitamin C levels were significantly reduced in potentially malignant disorders including OSMF. This is in accord with the study of R Guruprasad in 2014. In his study the serum vitamin C was significantly reduced in oral submucous fibrosis compared to control group. Though in this study there was no significant association of vitamin C levels and clinical staging of OSMF (Guruprasad, 2014). There was no significant difference in salivary vitamin C levels in the histological grading of oral cancer and in degree of dysplasia, which is contradictory to the study of Balwant Rai in 2008. He concluded that the salivary levels of Vitamin C were significantly decreased in patient with advanced grade of oral cancer (Balwant Rai, 2008). Even though the mean vitamin C levels in oral cancer were less when compared to potentially malignant disorders, they were not statistically significant. This study suggests that there is no correlation between degree of dysplasia and vitamin C levels in potentially malignant disorders. Also there was no much significant difference in the salivary levels of vitamin C between males and females in oral cancer and potentially malignant disorders. In control group the mean levels of salivary vitamin C were less in females.

When salivary vitamin C levels were compared with age group, there were highly significant results above the age of 40 years. The mean salivary levels of vitamin C were very much reduced in oral cancer and potentially malignant disorders when compared with control group. These results again favor the role of vitamin C in initiation and promotion of oral cancer. When control group is taken alone, there was no significant difference in vitamin C levels in different age groups. This suggests that there may be no significant relationship between vitamin C levels and age. The same is for oral cancer and potentially malignant disorders. But as the age increases there is a strong relationship between reduced vitamin C levels and incidence of oral malignant and potentially malignant disorders. In our study the salivary levels of vitamin C were reduced in smokers when compared to the non smokers in the control group. This is in favor of Vitamin C, is a known antioxidant which plays a vital role in many physiological functions of body including prevention of cancer. In our study the results showed that there may be strong relation with reduced vitamin C and oral malignant and potentially malignant disorders. Even though vitamin C levels cannot independently predict oral cancer, our study showed that vitamin C may play an important role in inhibiting initiation and promotion of malignant changes of oral cavity. Our study also suggests that vitamin C may not play a role in the progression of malignancy since there was no correlation between the levels of vitamin C and degree of dysplasia.

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