RESEARCH ARTICLE

Lipid Peroxidation and Antioxidant Vitamin Status in Oral Cavity and Oropharyngeal Cancer Patients

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Abstract

Background: This study was conducted to determine levels of lipid peroxidation and antioxidant vitamin status in patients with oral cavity and oropharyngeal cancer. Methods: The study group consisted of a total number of 80 subjects between the age 40-68 years, 40 with clinically and histopathologically proved cases of oral cavity and oropharyngeal cancer and 40 normal healthy, age and sex matched volunteers as controls. Levels of lipid peroxidation products as malondialdehyde (MDA) and antioxidant vitamins as vitamin A and vitamin C were estimated and compared between the two groups. Results: There was a statistical significant difference in the mean MDA, plasma vitamin A and vitamin C in the oral and oropharyngeal cancer patients compared with the healthy controls (p<0.0001). Conclusions: Lipid peroxidation (MDA) is higher and plasma antioxidant vitamins like vitamin A and vitamin C were lower in oral cavity and oropharyngeal cancer patients than healthy controls.

Keywords: Oral cavity and oropharyngeal cancer - lipid peroxidation - malondialdehyde - vitamin A - vitamin C

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Introduction

In the head and neck region, two of the most common types of cancer are cancer of the oral cavity and cancer of the oropharynx. The oral cavity includes the lips, buccal mucosa (lining of the lips and cheeks), gingiva (upper and lower gums), front two-thirds of the tongue, floor of the mouth under the tongue, hard palate (roof of the mouth), and the retromolar trigone (small area behind the wisdom teeth). The oropharynx begins where the oral cavity stops. It includes the soft palate at the back of the mouth, the part of the throat behind the mouth, the tonsils, and the base of the tongue.

The mucosal surface of the oral cavity and entire oropharynx is exposed to alcohol and tobacco related carcinogens and is at risk for the development of a premalignant or malignant lesion. Recent studies show that usage of tobacco may be the leading cause of oral cavity and oropharyngeal cancer development and it is believed that smokers are more likely to develop oral cancer than nonsmokers. Carcinogens, which are present in high concentration in tobacco and its products and are also the leading cause of cancer in lungs, esophagus and several other organs (Masthan et al., 2012). More than 400,000 cases of oral and pharyngeal cancers diagnosed annually, with almost two-thirds of them found among male (Ferlay et al., 2010). Their incidence has increased in most countries over the last four decades (Posner, 2003). Among males, oral and pharyngeal cancer is the eighth most common cancer on a global level, and the third most common in certain parts of Asia (Avraham et al., 2012). However a population-based study showed an incidence of oral cancers and its trend in Khon Kaen significantly higher in females than males (Vatanasapt et al., 2011).

Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation (Harper's Biochemistry. 25th ed. 2000) in which many damaging aldehydes are formed particularly malondialdehyde (MDA), propanedial, 4-hydroxynonenal (4-HNE), etc. MDA is a major metabolite of arachidonic acid. It is well known that MDA serves as a reliable marker of free radical-mediated lipid peroxidation. It is one of the important indicators of free radical-mediated tissue injury. It participates in a variety of chemical and biological reactions including covalent binding to protein, RNA, and DNA. The endogenous formation of MDA during intracellular oxidative stress and its reaction with biologically important macromolecules makes MDA-DNA adducts a suitable biomarker of endogenous DNA damage (Zhang, 2002). Lipid peroxides are also reduced to fatty acids by reaction with vitamin E, forming the relatively stable tocopheroxyl radical, which persist long enough to undergo reduction back to tocopherol by reaction with vitamin C at the surface of the cell or lipoprotein (Harper's Illustrated Biochemistry, 2009).

Carotenoids have long been considered antioxidants because of their capacity to scavenge free radicals. Carotenoids protect lipids against peroxidation by

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quenching free radicals and other reactive oxygen species, notably singlet oxygen species. β– Carotene displays an efficient biological radical trapping antioxidant activity through its inhibition of lipid peroxidation induced by xanthine oxidase system (Loft et al., 1996). Vitamin C plays a vital role in antioxidant defense. It acts as a scavenger of free radicals and impedes the detrimental chain reactions triggered by the free radicals which would otherwise culminate in tissue damage leading to oxidative stress (Ames, 1993). It has been said that antioxidant nutrients may be utilized to a greater extent in oral cancer patient to counteract free radical mediated cell disturbances, resulting in a reduction in antioxidant level (Reza et al., 2009).

Patients with oral cancer show elevated levels of lipid peroxidation accompanied by antioxidant depletion (Nagini, 2001; Khanna et al., 2005) reported that the generation of oxygen free radicals can be prevented or scavenged by a host of antioxidant defence mechanisms. The oral cancer model has therefore become an ideal model for studies on oxygen free radical induced carcinogenesis because tobacco consumption exposes the oral host antioxidant defense mechanisms including non enzymatic antioxidants likes betacarotene,ceruloplasmin, vitamin C and vitamin E. The plasma levels of MDA were higher and the levels of SOD, vitamin A, vitamin C and ceruloplasmin were lower in the head and neck cancer patients as compared to those in the healthy controls (Malathi et al., 2011). Serum levels of vitamin A, C and E in oral cancer patients were significantly lower than those of healthy volunteers and the risk of oral cancer was higher in patients with low serum antioxidant vitamins (Lawal, et al., 2012). Clinical studies in the United States (Zheng et al., 1993) and Japan (Nagao et al., 2000) also found lower serum antioxidant vitamins in oral cancer patients compared with normal population.

The aim of this study was to examine level of lipid peroxidation and antioxidant vitamin status in patients with oral cavity and oropharyngeal cancer patients by evaluation of the serum levels of lipid peroxidation products as malondialdehyde (MDA) and antioxidant vitamins as vitamin A and vitamin C and compare with those of healthy controls.

Materials and Methods

The study group consisted of a total number of 80 subjects between the age 40-68 years. Out of 80 study group 40 were clinically and histopathologically proved cases of oral cavity and oropharyngeal cancer, attending the Department of oncology of Father Muller Medical College Hospital Mangalore, India and 40 were normal healthy, age and sex matched volunteers or controls. Subjects having any underlying systemic diseases, under chemotherapy, radiotherapy and antioxidant supplementation were not included in this study. Appropriate ethical clearance was obtained from the institution.

In all selected individuals about 6 ml of blood was collected in EDTA tube from large peripheral vein with aseptic precautions after obtaining informed consent. Plasma was separated after centrifugation at 3,000 rpm for 10 min. MDA was estimated in RBC hemolysate as thiobarbituric acid reactive substances (Ohkawa et al., 1979). MDA reacts with thiobarbituric acid at 100^0c in acidic medium to form pink coloured complex. The colour intensity of MDA-TBA complex was measured at 535 nm. MDA concentration was calculated using the molar extinction coefficient of MDA-TBA complex. [$1.56x10^5$L $mol^{-1}.cm^{-1}$]. The plasma levels of vitamin A (retinol) were assayed (Bessey et al., 1946) after extraction into heptane Retinol has an absorption peak at 327 nm. Proteins get precipitated on addition of ethanol and concentration of retinol determined by reading absorption of heptanes extract of retinol at 327 nm. Vitamin C (Ascorbic acid) was oxidized by cupric ions to form dehydroascorbic acid, which reacts with acidic 2,4-dinitrophenylhydrazine to form a red bishydrazone. The colour intensity was measured at 520 nm (Omaye et al., 1979).

The collected data obtained were analyzed using the Statistical Package for the Social Sciences, version 15.0 (SPSS15). Differences between the two groups were analyzed for statistical significance using the Student 't' test (independent 't' test). Results were presented as mean±standard deviation. A p-value of 0.05 or less was considered statistically significant.

Results

The results obtained in the present study were from total number of 80 subjects out of which 40 were healthy controls and 40 oral cavity and oropharyngeal cancer cases. Among 40 controls between age group of 40-68 years (49.28±9.785), 29 (72.5%) were men and 11 (27.5%) were women while among 40 oral cavity and oropharyngeal cancer cases between age group of 40-68 years (52.60±8.536), 31 (77.5%) were men and 9 (22.5%) were women (Table 1)

There was a statistical significant difference in the mean MDA, plasma vitamin A and vitamin C in the oral and oropharyngeal cancer patients compared with the healthy controls.($p<0.0001$) (Table 2)

Table 1. Sex and Age Distribution of Study Groups

		Group		Total
		Controls	Cases	
Sex	M	29 (72.50%)	31 (77.50%)	60 (75%)
	F	11 (27.50%)	9 (22.50%)	20 (25%)
Total		40 (100%)	40 (100%)	80 (100%)
Age (years)	Mean ±SD	49.28±9.785	52.60±8.536	50.94±9.160
Range (years)		40-68	40-68	40-68

Table 2. Comparison of MDA, Plasma Vitamin A and Vitamin C Levels in Healthy Controls and Head and Neck Cancer Patients (n=40)

Parameters	Group	Mean±SD	p value
MDA	Controls	486.08±110.22	<0.0001
nM/dL	Cases	1827.00± 254.38	HS
Vitamin A	Controls	47.85±8.93	<0.0001
µg/dL	Cases	26.20±9.24	HS
Vitamin C	Controls	1.01±0.27	<0.0001
mg/dL	Cases	0.343±0.11	HS

Discussion

In the present study mean MDA was significantly higher in oral cavity and oropharyngeal cancer cases compared with healthy controls and mean plasma vitamin A and vitamin C were significantly lower in oral and oropharyngeal cancer cases compared with healthy controls.

Increased levels of MDA in oral cavity and oropharyngeal cancer patients in our study confirm an increased lipid peroxidation and oxidative stress in these patients which might be due to the interaction of various carcinogenic agents, generating free radicals to a greater extent in these patients beyond their defending power or may be due to poor antioxidant system existing in these individuals. Byproducts of lipid peroxidation cause marked alteration in the structural integrity and function of cell membranes. Enzymatic and non-enzymatic antioxidants scavenge lipid peroxidation by products formed under physiological and pathological conditions. Thus, the observed decrease in, vitamin A and vitamin C in oral cavity and oropharyngeal cancer patients can be due to utilization of these antioxidants by affected tissues or in combating the excessive oxidative stress in circulation.

Increased lipid peroxidation product MDA and decreased antioxidant vitamins like vitamin A and vitamin C have been reported in various pathological conditions including oral cavity and oropharyngeal cancer patients (Nisha et al., 2008; Malathi et al., 2011; Nidarsh et al., 2012). Our results support these observations.

In conclusion, lipid peroxidation (MDA) is higher and plasma antioxidant vitamins like vitamin A and vitamin C are lower in oral cavity and oropharyngeal cancer patients than healthy controls. Further studies are required to investigate the antioxidant and lipid peroxidation status in oral cavity and oropharyngeal cancer patients with different stages of disease.

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