Assessment of serum antioxidant levels in oral and oropharyngeal carcinoma


Original Articles

Abstract
Oral cavity and the oropharynx are the two most common sites of cancer in the head and neck region. The exposure of mucosal surface of the oral cavity and entire oropharynx to alcohol and tobacco-related carcinogens increases the risk for developing premalignant or malignant lesions. The purpose of this study is to assess the serum levels of total antioxidant capacity, vitamin C and malondialdehyde in confirmed cases of oral and oropharyngeal squamous cell carcinoma and their correlation in healthy subjects.

Keywords: Oral carcinoma; Oropharyngeal carcinoma; Total antioxidant capacity; Vitamin C; Malondialdehyde

Introduction
Cancer of the oral cavity accounts for approximately 200,000 annual deaths worldwide and around 46,000 deaths in India. Tobacco is the primary etiological factor and other factors include alcohol, genetic predisposition and a diet lacking micronutrients. In India, there is a striking incidence of oral cancer, accounting for 30-40% of all cancers. Males, particularly over 40 years, are affected twice when compared to females. 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 premalignant or malignant lesions.

Reactive oxygen species (ROS) and other free radicals are generated in all aerobic cells through normal metabolism. Exposure to high oxygen concentrations and to exogenous oxidants like ozone, asbestos fibers, cigarette smoke, radiation, and certain drugs increases the production of ROS. Oxygen free radicals, playing a role in the development of cancer, damage the cells, especially the DNA. Byproducts of lipid peroxidation such as malondialdehyde (MDA) have been shown to cause profound alterations in the structural organization and functions of the cell membrane including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids. The most important characteristic of lipid peroxidation is to cause a considerable DNA-MDA adducts by interacting with cellular DNA.

Human body has developed several endogenous antioxidants like superoxide dismutase, catalase, glutathione peroxidase and smaller molecules like vitamin C, vitamin E, reduced glutathione that scavenge the free radicals and inhibit the neoplastic process. Any changes in one of these systems may break this equilibrium and cause cellular damages and ultimately malignant transformation.

Antioxidants terminate the chain reaction caused by free radicals of oxidation reaction, thereby preventing cell damage or death of the cells. Insufficient levels of antioxidants or inhibition of antioxidant enzymes causes oxidative stress damage or cell death. It has been suggested that negative effects of nicotine are reversed by antioxidants. Vitamin C is effective in protecting against oxidative damage in tissues and also in suppressing formation of carcinogens like nitrosamines. Many studies have shown that vitamin C intake confer protective effects against cancer.

Discovery of tumor markers including tumor-associated antigens has contributed to the early detection of cancer. The markers including enzymes, hormones, antigens...
and proteins, which are present in higher concentrations only in the body fluids or tissues of cancer patients, are proportional to the tumor burden. The assessment of novel markers has opened up the scope for studying other molecular interactions like oxidative damage for management of malignancies. The present study is aimed at assessing the antioxidant level in patients with oral and oropharyngeal squamous cell carcinoma.

Materials and methods
The study involved subjects between 35-60 years of age, diagnosed with oral and oropharyngeal squamous cell carcinoma in the ENT department at a tertiary health care center in Mangalore. The study was approved by the institutional ethical committee. Informed and written consents were obtained from all the study subjects. The study included same number of age- and sex-matched healthy individuals as controls.

Five ml of blood was collected by venipuncture and transferred to sterile vials without anticoagulant by following aseptic precautions. The serum was separated by centrifugation at 1500 rpm for 15 minutes and stored at 4°C. Estimation of the total antioxidant capacity (TAC) was done by phosphomolybdenum method, MDA by spectrophotometric method and vitamin C by dinitrophenylhydrazine (DNPH) method.

Results
Out of the 15 patients with oral and oropharyngeal squamous cell carcinoma, 10 (67%) were males and 5 (33%) were females. The age group of the patient ranged from 35 to 60 and maximum number of subjects belonged to the age group of 51-60 years. Youngest was 35 years of age and the oldest was 60 years. The study revealed that among 15 patients, 14 (93.33%) had the habit of tobacco chewing. Ten patients (66.67%) were smokers and 9 (60%) had the habit of alcohol intake. The most common site of malignancy was tongue (46.67%), followed by buccal mucosa (40%). Out of 15 cases, 14 had oral cavity lesions and one had oropharyngeal malignancy (tonsil).

In the study group, all the subjects had ulceroproliferative type of morphology with squamous cell carcinoma. Most of the patients presented as stage IV type of oral squamous cell carcinoma (OSCC, 60%). Most of them had moderately differentiated squamous cell carcinoma (73.33%), followed by well-differentiated squamous cell carcinoma (20%), and poorly differentiated squamous cell carcinoma (6.67%). Fifteen age- and sex-matched healthy individuals, without any addictions or systemic diseases, were selected as controls.

Table 1: Estimation of TAC, vitamin C and MDA levels in patients and controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>TAC (Mean±S.D) (mM/L)</th>
<th>Vitamin C (Mean±S.D) (mg/dl)</th>
<th>MDA (Mean±S.D) (µM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n=15)</td>
<td>0.9354±0.19417</td>
<td>0.5062±0.8244</td>
<td>2.2588±0.60264</td>
</tr>
<tr>
<td>Controls (n=15)</td>
<td>1.2032±0.17078</td>
<td>1.2643±0.35471</td>
<td>0.8661±0.19481</td>
</tr>
</tbody>
</table>

P value <0.05 was not statistically significant in TAC, whereas significant in vitamin C and MDA.
significantly elevated levels of mean serum MDA in all oral cancer patients as compared to the controls (P < 0.05) (Table 1).

Discussion
The present study evaluated the total antioxidants capacity, MDA and vitamin C in patients with histologically confirmed oral and oropharyngeal cancer. As in the earlier studies on oral cancers, most of the patients were in the 5th decade of life. Among the subjects, 93.33% were tobacco chewers; 66.67% were smokers and 60% were alcohol consumers.

The study by Bhat et al. has found that 58% patients were smokers, 52% were consuming smokeless tobacco in the form of pan or gutkha, which is a combination of arecanut, tobacco and slaked lime with sweet flavor; 37% of patients were consuming alcohol, 44% were consuming alcohol as well as tobacco, either smoking or chewing. Smoking and alcohol consumption were seen only in males.

The study by Patel et al. has reported that the anterior 2/3 of the tongue is the most common site (23.02%) of oral cancer, followed by the posterior 1/3 (19.64%), alveolus, lips and cheeks. Type et al. have found the tongue as the most common site (52%), followed by cheek (26%), alveolus (10%), palate (4.5%), lip (2.3%) and floor of mouth (1.9%). Durazzo et al. have found that 55.6% of patients had cancer of the tongue and floor of mouth. It has been observed in various studies that anatomically more anterior parts (buccal mucosa, anterior 2/3 of the tongue, alveolus, lips, and base of tongue) are the frequently involved sites for oral and oropharyngeal malignancies. This could be due to the long duration of contact of these site with the carcinogens in tobacco and alcohol.

In the current study, 60% of the subjects had stage IV squamous cell carcinoma, followed by around 13% each with stage III, stage II and stage I. Majority of patients had rural background and sought medical consultation only at later stages.

Plasma levels of antioxidants are reliable indicators of the antioxidant status, because they reflect the bioavailability as well increased utilization to counter lipid peroxidation. Furthermore, antioxidants levels in plasma are also influenced by life style factors such as diet and tobacco. Korde et al. have conducted a study on enhanced nitrosative and oxidative stress with decreased TAC in 30 patients with oral precancer and same number of subjects with OSCC. Decreased TAC was noted in the serum of OSCC when compared to the control. In our study TAC mean value in healthy individual is 1.2032 mM/L with SD of ±0.17078, whereas mean value in OSCC patients was 0.9354mM/L with SD of ±0.19417. Though the absolute values of TAC were low in patients with OSCC, the values were not found to be statistically significant. This might be due to small sample size of the study.

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 result in tissue damage leading to oxidative stress. Priya et al. have studied the oxidant/antioxidant status in patients with oral squamous cell carcinoma in tissue and plasma. The plasma level of vitamin C was found to be diminished when compared to the healthy controls. Malathi et al. have studied the role of oxidative stress and the effect of radiotherapy on the plasma oxidant-antioxidant status in head and neck cancer. The study has reported reduced plasma levels of vitamin C in the head and neck cancer patients when compared to those in the healthy controls. These parameters showed significant changes after radiotherapy, as indicated by higher levels of vitamin C in the plasma of the cancer patients.

In the present study, mean value of vitamin C in oral cancer patients was 0.5062 nmol/ml with SD of ±0.8244 nmol/ml, whereas healthy individuals had 1.2643 nmol/ml with SD of ±0.35471 nmol/ml, which was higher than oral cancer patients. Overall the values were found to be statistically significant. A possible mechanism for the protective effect of vitamin C is the inhibition of nitrosamine formation from secondary and higher amines in combination with nitrite. 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.

The MDA value in the blood is the measure of the ability of the body to handle oxidative stress. Patait et al. conducted a similar study on estimation of serum MDA before and after radiotherapy in oral squamous cell carcinoma patients undergoing antioxidant therapy. The corresponding mean±SD levels of serum MDA noted in the control group and the study group were 0.3084±0.10167 μM/L and 0.598± 0.1609 μM/L. In the present study, the MDA mean±SD value in oral cancer patients was
2.2588±0.60264 µM/L, while the value noted in healthy individuals was 0.8661±0.19481µM/L, which was lower than the oral cancer patients. Overall the values were found to be statistically significant.

High levels of serum MDA in oral cancer patients directly reflects increased oxidative stress and lipid peroxidation, which might be due to the interaction of various carcinogenic agents generating free radicals beyond their defending power or may be due to poor antioxidant system existing in these individuals. Thus measurement of serum MDA determines an extent of lipid peroxidation and antioxidant status.

**Conclusion**
The present study was conducted in 15 patients with oral oropharyngeal malignancy and 15 age- and sex-matched healthy individuals. The study has shown that the plasma level of vitamin C was lesser in oral and oropharyngeal squamous cell carcinoma patients when compared to healthy individuals (P <0.05). Statistically significant increase in the level of MDA, a marker of oxidative stress, was noted in patients with oral and oropharyngeal squamous cell carcinoma than normal healthy individuals (P <0.05). Though the absolute values of TAC were low in patients with OSCC, they were not statistically significant. This might be due to small sample size of the study. Further study involving a large sample size is required to ascertain the study findings.

**Competing interests**
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**References**
19. Prieto, Manuel, P. and Miguel, A. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdnum complex: specific application to the determination of vitamin E. Analytical biochemistry 1999; 259: 337-41.
23. Bhat SP, Naik RCN, GK Swetadri, D'souza H, Jayaprakash CS, Bhat V. Clinicopathological Spectrum Of Malignancies Of Oral


