



## Original Research Article

## Status of lipid peroxidation and antioxidation in maxillary and vocal cord carcinoma

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## ABSTRACT

**Introduction:** Present study was conducted to investigate the oxidative, stress by measuring the lipid peroxidation marker Malondialdehyde (MDA) in maxillary cancer and vocal cord cancer. And, to study the role of non-enzymatic antioxidants like ascorbic acid (vitamin C) and tocopherols (vitamin E) in maxillary cancer and vocal cord cancer.

**Materials and Methods:** Total 100 patients were selected.

**Inclusion Criteria:** Study included Maxillary and Vocal cord patient.

**Exclusion Criteria:** The exclusion criteria were male factor chronic disease like chronic heart disease (CHD), hypertension, ischemic heart disease, diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), other major systemic illness in different age group. MDA estimation done by Thiobarbituric acid reactive substance (TBARS) (Buege and Aust, 1978) method and vitamin C determined by the method of Natelson, (1971) and Vitamin E by Baker and Frank method.

**Results:** Majority of patient were between age group 41 to 60 years. Here, Mean MDA, Vitamin-C and E Level in case and control was  $7.09 \pm 2.15$  and  $2.3 \pm 1.06$ ;  $0.225 \pm 0.067$  and  $0.998 \pm 0.19$  and  $0.456 \pm 0.155$  and  $1.02 \pm 0.167$  respectively.

**Conclusion:** Maxilla and vocal cord cancer is a major cause of morbidity and mortality in both developing and developed countries. We found an associations of vitamin C (Ascorbic acid), vitamin E (Tocopherol) and MDA with oral cancer.

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### 1. Introduction

Oral cancer is the 8<sup>th</sup> most common cancer in the world among men. Oral cancer, which includes cancers of lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses, and pharynx, can be life threatening if not diagnosed and treated early.<sup>1</sup> The most important etiology factors associated with oral cancer are tobacco chewing with betel quid, tobacco smoking and alcohol consumption are responsible for oral cancer in India. Squamous cell carcinoma (SCC) of the oral cavity is responsible for considerable morbidity and mortality in India where 60,000 new cases of oral cancer are reported to occur every year.<sup>2</sup>

Maxillary cancer is the predominant neoplastic tumor that occurs in the oral cavity.<sup>3</sup> Vocal cord carcinoma is not an uncommon disease in men. Vocal cord cancer an estimated 13,560 new cases and 3640 deaths in every year in world.<sup>4</sup> Most cancer expert agree that vocal cord cancer likely starts as small areas of abnormal cells that undergoes sequential changes that ultimately leads to the development of cancer.<sup>5</sup>

Although the causes of the high incidence of oral cancer is poorly understood, epidemiological, experimental and clinical studies, suggest that oxidative stress (OS) is one of the parameters in explaining cancer development and progression.<sup>6</sup>

It has long been recognized that high levels of free radicals or reactive oxygen species (ROS) can inflict direct damage to lipids. Lipid peroxidation or reaction of

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oxygen with unsaturated lipids produces a wide variety of oxidation products.<sup>7</sup> Deleterious effects of ROS and lipid peroxidation (LPO) products are counteracted by antioxidant (AO) defense system.<sup>8</sup>

As a result of high ROS level secondary product formed during and lipid peroxidation are malondialdehyde (MDA), propanol, hexanal, and 4-hydroxynonenal (4-HNE). MDA appears to be the most mutagenic product of lipid peroxidation, whereas 4-HNE is the most toxic.<sup>7</sup>

It is possible to measure the extent of peroxidative damage by estimating the stable end products of lipid peroxidation such as Malondialdehyde (MDA). MDA level is commonly known as a marker of oxidative stress and the antioxidant status. The physiological concentration of these products are low, however higher concentrations correspond to pathological situations.<sup>9</sup>

The body has designed several physiological responses to oxidative stress including counterbalancing such as enzymes and variously functionalized molecules like ascorbic acid and  $\alpha$ -tocopherol etc. that effectively neutralize these damaging species. The antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions.<sup>9</sup>

Vitamin C is an essential nutrient and highly effective antioxidant for humans. Vitamin C acts as an electron donor for important enzymes. Vitamin C is act as antioxidant and has been shown to inhibit the formation of nitrosamines. It also acts on the immune system, thereby reducing the risk of cancer. Vitamin E refers to a group of compounds that include both tocopherols and tocotrienols. The most important function of vitamin E has been suggested to be in cell signaling. Vitamin E has a role in inhibiting cancer via its action as an antioxidant, as well as its potential effects on selenium. It reduces nitrite, thereby inhibiting the production of carcinogenic nitrosamines and nitroso amides and expression of certain oncogenes. Vitamin E can neutralize reactive oxygen species, may reduce oxidative DNA damage, genetic mutations and also enhance host immunological functions. These reactions may help to protect against carcinogenesis. Vitamin E levels were observed to be significantly decreased in cancer patients.<sup>10</sup>

An impairment of the antioxidant defense system has been implicated in many diseases including cancers, and the activities of non-enzymic antioxidants have shown different patterns during neoplastic transformation.<sup>11</sup> Therefore the present study was designed to examine the lipid peroxidation status by estimating MDA and the antioxidant status through vitamin C and vitamin E in men with oral cancer.

## 2. Materials and Methods

Total study population of 100 were selected for the study and divided into two groups.

### 2.1. Inclusion criteria

Study included Maxillary and Vocal cord patient.

### 2.2. Exclusion criteria

The exclusion criteria were male factor chronic disease like chronic heart disease (CHD), hypertension, ischemic heart disease, diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), other major systemic illness in different age group.

Details about name, age, urban/rural residence, height, weight were taken. Other medical history including obstetrics, diabetes, blood pressure, other clinical manifestation was noted. Patients and controls with a history of smoking, alcoholism, tobacco chewing habit and other risk factors taking drugs for any other reason were not included. Informed consent was obtained from each patient before sample collection. The study was approved by GMCH ethical committee.

10 ml blood sample was collected in a plain vial by vein puncture and allowed the blood to clot at room temperature and centrifuged at 3000 rpm for 10 min. The serum separated into proper aliquots and analyzed for test.

MDA estimation done by Thiobarbituric acid reactive substance (TBARS) (Buege and Aust, 1978) method and vitamin C determined by the method of Natelson, (1971) and Vitamin E by Baker and Frank method.

## 3. Results

Present study included 50 patients of maxilla & vocal cord cancer and 50 controls, who attended oncology unit of Geetanjali medical college and hospital.

Here, majority of patient were between age group 41 to 60 years (74%) followed by age group 20-40 years (26%). The persons included in controls were between of age group 41-60 years (54%) and 20-40 years (46%). 29 (58%) patients of the study were male while rest of 21 (42%) were female. In control group also, 35 (70%) were male and 15 (30%) were female. Mostly patients from rural areas.

Figure 1 shows the personal history of patients. Here, 54%, 56% and 73% were alcoholics, smokers and tobacco chewer respectively and 46%, 44% and 26% were non-alcoholics, non-smokers and non-tobacco chewer.

Table 1 shows the level of MDA, Vitamin-C and Vitamin-E in cases and controls and we found that difference was statistically significant as p-value in less than 0.05.

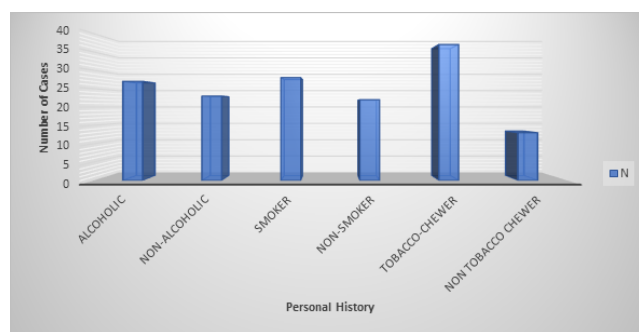
Table 2 shows the difference of MDA, Vitamin-C and E level in two age group and we found that level of MDA in two groups were statistically significant but the level of antioxidant was not statistically significant. This shows that lipid peroxidation increases with age but the age does not have significant effect of activity of antioxidant. Figure 2 Showing a negative linear Pearson correlation ( $r = -0.73$ ;  $R^2 = 0.5034$ ) between MDA and Vitamin C in oral cancer

**Table 1:** MDA, Vitamin-C and Vitamin-E level in cases and controls.

Parameters	Case	Control	P-value
	Mean ± SD	Mean ± SD	
MDA	7.09 ± 2.15	2.3 ± 1.06	< 0.0001
Vitamin-C	0.225 ± 0.067	0.998 ± 0.19	< 0.0001
Vitamin-E	0.456 ± 0.155	1.02 ± 0.167	< 0.0001

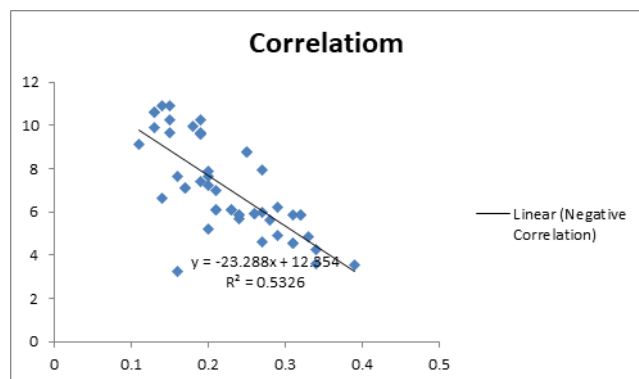
**Table 2:** MDA, Vitamin-C and E level in two age group.

Parameters		21-40 years (N=13)	41-60 years (N=23)	P-value
MDA	Cases	8.53± 1.59	6.87± 2.28	0.0001
	Controls	2.35± 1.04	2.3± 1.04	0.7033
Vitamin-E	Cases	0.49± 0.22	0.44±0.12	0.8921
	Controls	1.03± 0.16	1.02±0.17	0.9863
Vitamin-C	Cases	0.50± 0.25	0.60± 0.27	0.6932
	Controls	1.03± 0.21	0.97± 0.16	0.4301



**Fig. 1:** Distribution showing personal history of cases

patients.



**Fig. 2:** Correlation between MDA and Vitamin-c

**4. Discussion**

The present study was carried out in Biochemistry and Oncology department of Geetanjali Medical College and Hospital and it included a total of 50 newly diagnosed maxilla and vocal cord cancer patients and 50 controls. We were determined the level of MDA, Vitamin C and Vitamin

E in the serum of all the patients as well as in controls and finally the results of maxilla and vocal cord cancer patients were compared with that of controls.

Maxilla and vocal cord cancer are a complex and multifactorial disease. Epidemiological research has shown the positive benefit of vitamin C, vitamin E and nutrition supplementation on the antioxidant enzyme defense system hence prevention of oral carcinogenesis in patients.<sup>12</sup>

In our study, 26% cases were from age group 21-40 years and 74% cases from age group 41-60 years. In line with this, Goncalves et al., conducted a study in which 32.6% cases were in 20-45 years and 43% cases were in group 30-40 years.<sup>13</sup>

The present study showed that incidence and mortality is high in rural areas as compared to urban areas. Here, 70% maxilla and vocal cord cancer cases were from rural areas where only 30% cases were from urban area. Similarly, our study is correlated with the study of, according this cancer mortality rates are also significant in rural regions, as compared to urban areas, where cancer treatment facilities are scarce. Poor individuals are also at a higher age-specific mortality risk than are affluent people.<sup>14</sup>

Feller and Lemmer studied that oral squamous cell carcinoma more frequently affects men than women (M: F = 1.5:1) most probably because more men than women indulge in high-risk habits. The probability of developing oral SCC increases with the period of exposure to risk factors, and increasing age adds the further dimension of age-related mutagenic and epigenetic change.<sup>15</sup> Studies on the relationship between risk factors and maxilla and vocal cord cancer suggest that diet, tobacco chewing, smoking and alcohols were suggested as risk factors.<sup>1</sup> But in our study we found only 74% cases of tobacco chewer, 54% cases of alcoholics, 56% cases of smoker. There was a significant difference in tobacco chewing, smoking, alcohol intake habit and effect of diet between two groups.

Oxidative stress and impaired antioxidant system have been proposed as a potential factors involved in

the pathophysiology of diverse disease states, including carcinogenesis.<sup>8</sup> Increased levels of lipid peroxides in the plasma and erythrocytes were reported on for patients with maxilla and vocal cord cancer.<sup>2</sup> Lipid peroxidation has been implicated in the pathogenesis of various diseases including cancer.<sup>13</sup> In line with this, the present study shows that serum MDA level is significantly elevated in maxilla and vocal cord cancer cases. The mean of serum level of MDA in cases was 7.09 with standard deviation of 2.15 as compared to controls having mean 2.3 with standard deviation of 1.06. A comparison for MDA level in serum was also made between different age groups using unpaired t-test and found that a highest significant difference for lipid peroxidation is present in age groups 20-40 years in maxilla and vocal cord cancer patients). This is shown by a high significance level <0.05 with a p-value of 0.0001 in age group 20-40 years as compared to other age groups at a confidence level of 95%.

Extensive work has been carried out on the relationship between free radical activity, antioxidants scavenging of free radicals, and cancer of the maxilla and vocal cord. Impaired antioxidant status in the carcinoma of the maxilla and vocal cord was also demonstrated. The decrease in antioxidant enzyme activity may be attributed reduced synthesis of said enzymes in the tumor tissue. The elevated lipid peroxidation level in turn may be due to extensive tissue damage or a decrease in the efficacy of the antioxidant defense mechanism.<sup>11</sup> Antioxidant vitamins, such as  $\alpha$ -carotene,  $\beta$ -carotene, vitamin E, and vitamin C could act as efficient scavengers of free radicals and oxidants to prevent free-radical damage to DNA. Moreover, if the free radicals and oxidants were not neutralized by antioxidant molecules, inflammatory processes could lead to extensive damage to DNA proteins. It has also been hypothesized that possessing antioxidant properties may protect the immune system from oxidative damage; enhance immune responsiveness.<sup>16</sup> Antioxidant vitamins are an important component of human non-enzymatic antioxidant defense.<sup>13</sup> In this study, we show that serum vitamin C and vitamin E level is significantly decreased in patients with maxilla and vocal cord cancer. The mean of serum level of vitamin C and vitamin in cases were 0.225 with standard deviation of 0.067 and mean 0.456 with standard deviation 0.155 respectively, as compared to controls having mean 0.998 with standard deviation of 0.19 and mean 1.02 with standard deviation of 0.167 respectively. A comparison for vitamin C and vitamin E in serum were also made between different age groups using unpaired t-test and found that a highest significant difference for suppressed antioxidant is present in age group, 40-60 years for vitamin C and 20-40 years for vitamin E is present in maxilla and vocal cord cancer patients. This is shown by a high significance level <0.05 with a p-value of 0.0001 in age group 40-60 years for vitamin C and 20-40 years for vitamin E as compared to other age groups at a confidence level of 95%.

The decreased levels of Vitamin-C may be associated with its action as antioxidant where it gets utilized. Its synergism with Vitamin-E helps in sparing of Vitamin-E, during this process Vitamin-C gets utilized which is seen as significant decline in plasma ascorbic acid. Negative correlation ( $r = -0.73$ ) between Vitamin-C and MDA was noted leading to the conclusion that free radicals are scavenged by ascorbic acid and thus it gets utilized.<sup>17</sup> Ascorbic acid is present on aqueous compartment and can reduce the tocopherol radical.<sup>18</sup> It can also help recycle oxidized vitamin E (Agarwal et al, 2005) In line with these studies, we also found a negative correlation ( $r = -0.73$ ) between MDA and vitamin C. We also calculated the Pearson correlation between MDA-Vitamin E and Vitamin C-Vitamin E, but we do not find a significant correlation between these parameters.

The results of the present study suggested that Vitamin C and vitamin E can be considered as biochemical marker of oxidative stress associated with cancer and can be recommended to patients to check or slowing down the progression of oral cancer. The etiology of oral cancer is multifactorial and various risk factors have been described. Deficiency of nutrients in developing countries can be a significant contributing factor in modifying the multistage process of carcinogenesis. In Indian population the low mean vitamin C and vitamin E levels in maxilla and vocal cord cancer patients as compared to the controls indicated their strong association with maxilla and vocal cord cancer.

## 5. Conclusion

Maxilla and vocal cord cancer are a major cause of morbidity and mortality in both developing and developed countries. Though many advancements have been done to improve early diagnosis and proper management of oral cancer, still the increasing incidence of oral cancer demands some new reliable and easy method for detection of oral cancer.

Till now, very few studies have been undertaken in India on the association between micronutrients and the risk of oral cancer. We therefore conducted a hospital-based case-control study to examine the associations of vitamin C (Ascorbic acid), vitamin E (Tocopherol) and MDA with oral cancer.

## 6. Source of Funding

None.

## 7. Conflict of Interest

The authors declare that there is no conflict of interest.

## References

1. Mehanna P, Goddard R. Bisphosphonate associated osteonecrosis: an unusual case. *Aust Dent J.* 2010;55:311-3. doi:10.1111/j.1834-7819.2010.01240.x.

2. Manoharan S, Kolanjiappan K, Suresh K, Panjamurthy. Lipid peroxidation & antioxidants status in patients with oral squamous cell carcinoma. *Indian J Med Res.* 2005;122(6):529–34.
3. Leiser Y, Yudovich K, Barak M, Naaj IE. The Management of Maxillary Squamous Cell Carcinoma—A Retrospective Study. *J Cancer Ther.* 2014;05(12):1065–71. doi:10.4236/jct.2014.512112.
4. Sheu T, Vock DM, Mohamed AS, Gross N, Mulcahy C, Zafereo M. Conditional Survival Analysis of Patients With Locally Advanced Laryngeal Cancer: Construction of a Dynamic Risk Model and Clinical Nomogram. *Sci Rep.* 2017;doi:10.1038/srep43928.
5. Hopkins J. Treatment of Vocal Cord Cancer with antioxidants. *Int J Pharma Bio Sci.* 2009;4.
6. Freitas M, Baldeiras I, Proença T, Alves V, Mota-Pinto A, Sarmento-Ribeiro A. Oxidative stress adaptation in aggressive prostate cancer may be counteracted by the reduction of glutathione reductase. *FEBS Open Bio.* 2012;2:119–28. doi:10.1016/j.fob.2012.05.001.
7. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog.* 2006;5:14.
8. Pejic S, Todorovic A, Stojiljkovic V, Kasapovic J, Pajovic SB. Antioxidant enzymes and lipid peroxidation in endometrium of patients with polyps, myoma, hyperplasia and adenocarcinoma. *Reprod Biol Endocrinol.* 2009;7:149.
9. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol.* 2005;3:28.
10. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv.* 2015;5(35):27986–28006. doi:10.1039/c4ra13315c.
11. Kierzenkowska CM, Kornatowska KK, Wozniak A, Drewna T, Wozniak B, Drewna S. The effect of brachytherapy on antioxidant status and lipid peroxidation in patients with cancer of the uterine cervix. *Cell Mol Biol Lett.* 2004;9:511–8.
12. Khanna R, Thapa PB, Khanna HD, Khanna S, Khanna AK, Shukla HS. Lipid peroxidation and antioxidant enzyme status in oral carcinoma patients. *Kathmandu Univ Med J.* 2005;3:334–9.
13. Gonçalves TL, Erthal F, Corte CLD, Müller LG, Piovezan CM, Nogueira CW. Involvement of oxidative stress in the pre-malignant and malignant states of cervical cancer in women. *Clin Biochem.* 2005;38(12):1071–5. doi:10.1016/j.clinbiochem.2005.09.008.
14. Mallath MK, Taylor DG, Badwe RA, Rath GK, Shanta V, Pramesh CS. The growing burden of cancer in India: epidemiology and social context. *Lancet Oncol.* 2014;15(6):205–12.
15. Feller L, Lemmer J. Oral squamous cell carcinoma : Epidemiology, clinical presentation and treatment. *J Cancer Ther.* 2012;3:263–8.
16. Guo L, Zhu H, Lin C, Che J, Tian X, Han S, et al. Associations between antioxidant vitamins and the risk of invasive cervical cancer in Chinese women: A case-control study. *Sci Rep.* 2015;5(1):13607. doi:10.1038/srep13607.
17. Naidu MSK, Suryakar AN, Swami SC, Katkam RV, Kumbar KM. Oxidative stress and antioxidant status in cervical cancer patients. *Indian J Clin Biochem.* 2007;22(2):140–4. doi:10.1007/bf02913333.
18. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am J Clin Nutr.* 1995;62(6):1315S–21S. doi:10.1093/ajcn/62.6.1315s.

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