EFFECT OF ORAL ANTIOXIDANT SUPPLEMENTATION ON LIPID PEROXIDATION DURING RADIOTHERAPY IN HEAD AND NECK MALIGNANCIES

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ABSTRACT

The objectives of the present study were to ascertain the variations in the serum levels of malondialdehyde and total antioxidant status, in head and neck malignancies with different stages, with and without oral antioxidant supplementation, before and after radiotherapy, and to validate the protective effects of an antioxidant supplementation during radiotherapy. The pretreatment values of serum malondialdehyde were significantly raised, while that of serum total antioxidant status were significantly declined in all the stages of head and neck malignancies, when compared with the healthy controls values ($P < 0.001$). A significant correlation was observed related to the studied parameters and different stages of the disease. The study suggests that an oral antioxidant supplementation during radiotherapy is an effective mode in reducing oxidative stress. Antioxidant supplementation during radiotherapy may serve as an adjuvant therapy in malignancies offering a protection to normal cells that may further reduce the risk of developing secondary cancers.

KEY WORDS

Antioxidant supplementation, Head and neck malignancies, Lipid peroxidation, Malondialdehyde, Radiotherapy, Total Antioxidant Status.

INTRODUCTION

It is well known that Reactive Oxygen Species (ROS) mediate cytotoxic tumor activity and antioxidants mediate anticancer activity, by modifying the cellular homeostatic redox balance. Lipid peroxidation is an indicator of free radical metabolism and oxidative stress in living beings. Malondialdehyde (MDA) a byproduct of lipid peroxidation (LPO), which inherent in carcinogenesis showed discrepancies in its serum values in radiation treated malignant cases of earlier research studies (1-4). Antioxidants are natural or synthetic substances that may quench the free radicals as soon as they are formed, so that the reactive oxygen species do not get the chance to damage the vital components of DNA or other cellular components. Human cells are equipped with both enzymatic

and non-enzymatic antioxidant defence mechanisms to minimize the cellular damage resulting from the interaction between the cellular constituents and the ROS. Ionizing radiations used in radiotherapy results in generating free radicals (5, 6), which brings oxidative damage to the cell membrane and its consequent role in the mechanism of apoptotic cell death (7-9). The present study was designed to ascertain the variations in the levels of serum MDA, a marker of oxidative stress that determines an extent of lipid peroxidation and concentration of serum total antioxidant status (TAS) an antioxidant parameter which reflects the antioxidant status, in most common head and neck malignancy cases with different stages, undergoing radiotherapy. The study was further planned to validate the effects of an oral antioxidant supplementation on lipid peroxidation, antioxidant status during radiotherapy in studied cases of head and neck malignancies.

MATERIALS AND METHODS

The study was carried out in 96 previously untreated, clinically and histopathologically proven head and neck malignancy cases, with early (I and II) and late (III and IV) stages (according

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to TNM system of cancer classification), referred and treated in the radiotherapy department of Krishna Hospital and Research Centre, Karad. Thirty cases of head and neck malignancies were randomly included in group I and supplemented with a combination of oral antioxidant; multivitamin and multimineral tablets once in a day for 35–40 days, during radiotherapy course (The contents of each tablet is given in Table). Sixty six malignancy cases of the study were considered under group II and were not supplemented with oral antioxidant tablets during radiotherapy. The patients were treated with the standard dose of radiations (60-66 Gy / 30-33 fractions for 6-7 weeks) depending upon the grade and stage of malignancy.

Sixty three healthy volunteers of Karad city served as healthy controls. The patients associated with diseases like rheumatoid arthritis, tuberculosis, gout, diabetes mellitus, pneumonia and myocardial infarction were excluded out from the study.

The first venous blood sample was collected from the patient before initiation of radiotherapy and the second venous blood sample after completion of radiotherapy course. Single blood samples were collected from healthy controls. The blood samples were allowed for clot retraction and later centrifuged at 3000 rpm for 10 minutes to obtain the serum. The estimations of serum MDA (10) and serum TAS (11) were carried out by simple, inexpensive, feasible and standard

**Table : Composition of Antioxidant A–Z Tablet
(Antioxidants + Multivitamin+ Multimineral)**

Contents	Therapeutic Concentrations
1 Vitamin – A (as acetate) IP	5000 IU
2 Vitamin – E (Acetate) IP	25 IU
3 Vitamin – C (Ascorbic acid) IP	100 mg
4 Thiamine mono nitrate IP	10 mg
5 Riboflavin IP	10 mg
6 Pyridoxine hydrochloride IP	3 mg
7 Cyanocobalamin IP	5 mg
8 Niacin amide IP	50 µg
9 Folic acid IP	1 mg
10 Calcium pantothenate IP	12.5 mg
11 Zinc oxide IP	15 mg
12 Cupric oxide	2.5 mg
13 Sodium selenate	60 µg
14 Manganese chloride	1.4 mg
15 Chromium chloride	65µg

laboratory methods and the readings of concentrations were read on Erba Chem 7 semi autoanalyzer. The study was carried out after obtaining clearance from the institutional ethics committee. Informed consents were obtained from all the studied subjects.

The study results were statistically analyzed with the program of SPSS for window version 10.0. The results were expressed as mean±SD ANOVA and Student's 't' test were applied for statistical comparison and significance.

RESULTS AND DISCUSSION

In randomly selected 96 head and neck malignancy cases with different stages, 84 cases were from 45-60 years of age group and 12 patients, were from 61-75 years of age group (Table 1). It was observed that the percentage of malignancy patients with late stages (73.96%) was higher than the early stages (26.04%).

Table 1: Age and stage wise distribution of head and neck malignancy cases

Age groups	Head and Neck Malignant cases		Total
	Early Stages (Stages I +II)	Late Stages (Stages III +IV)	
45-60 years	21	63	84
61-75 years	04	08	12
Total and Percentage	25 (26.04%)	71 (73.96%)	96 (100%)

The pretreatment mean serum MDA levels ($6.21 \pm 2.54 \mu\text{moles/L}$) of head and neck malignancy patients showed marked and significant increase, whereas the mean serum TAS values ($628.75 \pm 76.72 \mu\text{moles/L}$) of these patients showed statistically significant decrease, when compared with the values of healthy controls ($1.36 \pm 0.46 \mu\text{moles/L}$, $997.13 \pm 82.25 \mu\text{moles/L}$) respectively (Table 2). Before initiation of the treatment, the mean serum MDA levels with late stages of malignancy were higher than that of early stages, whereas the serum TAS values were lower in late stages than early stages; on comparison of these results it showed statistical significance ($P < 0.001$).

Effects of an oral antioxidant supplementation on oxidative stress during radiotherapy on studied groups of head and neck malignancy cases are depicted in Table 3 and 4. The post treated mean serum MDA of Group I patients showed statistically significant lower values ($P < 0.01$), whereas the group II study cases showed statistically significant higher values ($P < 0.001$). The mean serum TAS levels of group I cases

Table 2: Mean serum levels of malondialdehyde and total antioxidant status in healthy controls and pre treated cases of head and neck malignancies

Groups	Number	Serum MDA (μmoles/L)	Serum TAS (μmoles/L)
Healthy Controls	63	1.36 ± 0.46	997.13 ± 82.25
Head and Neck Malignant Patients	96	6.21 ± 2.54*	628.75 ± 76.72*
Early Stages	25	3.68 ± 0.61*	718.56 ± 46.95*
Late Stages	71	7.11 ± 2.36*	597.12 ± 57.76*

*P < 0.001; Values are mean±SD

showed statistically significant elevations than group II head and malignancy cases following radiotherapy (P<0.001).

Table 3: Mean serum malondialdehyde levels before and after radiotherapy in study groups of head and neck malignancies

Groups	No	Serum MDA (μmoles/L) BT	AT
Group I	30	6.39 ± 0.64	5.40 ± 0.95*
Group II	66	6.41 ± 2.51	8.20 ± 2.80**
Controls	63	1.36±0.46	

BT – Before Treatment; AT – After Treatment;
Values are Mean ± SD; * P < 0.01 and ** P<0.001.

Table 4: Mean serum levels of total antioxidant status before and after radiotherapy in study groups of malignancy patients

Groups	No	Serum TAS (μmoles/L) BT	AT
Group I	30	746.21 ± 49.07	788.03 ± 80.12*
Group II	66	742.29 ± 72.78	600.69 ± 71.16**
Controls	63	997.13 ± 82.25	

BT – Before Treatment; AT – After Treatment;
Values are Mean ± SD; *P < 0.01 and ** P<0.001.

Head and neck malignancies are the most common group of cancers that includes malignant tumors in the oral cavity, the oropharynx, nasopharynx and the larynx affecting the functions of eating and breathing. In the developing country like India the incidence and prevalence of head and neck malignancies is very high with common male: female ratio of 3:1 (12). Chewing tobacco and its products, smoking and alcoholism are the high risk factors for developing head and neck malignancies (13).

Ninety six head and neck malignancy patients and sixty three normal subjects included in the present study were in the range

of 45-75 years of age. The study groups comprised 90 % of males and only 10% of females. The predominant number of male patients suffered from head and neck malignancies indicates, that the habits of chewing tobacco and its products, smoking and alcoholism and other risk factors are more in males than females of Karad population. The high percentage of advanced stages of head and neck malignancy cases noted in the present study may be due to lack of awareness, illiteracy, ignorance and poverty, which might have not drawn these patients to the hospitals for early diagnosis and treatment.

The marked elevated levels of serum MDA and declined levels of serum TAS in head and neck malignancy patients before treatment, showed high statistical significance (P<0.001) when compared with healthy subjects. These high levels of serum MDA and low levels of serum TAS in head and neck malignant individuals directly reflects increased oxidative stress and lipid peroxidation in them, 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. The stages of the disease showed a significant positive correlation with the serum levels of MDA and a negative correlation with serum TAS values. Thus measurement of serum MDA and TAS determines an extent of lipid peroxidation and antioxidant status, in the studied cases that may be helpful in understanding the severity of the disease state and assessing the tumor stage of head and neck malignancies.

The present study evidenced, an oral antioxidant supplementation to malignancy patients undergoing radiotherapy had a beneficial effect in lowering the serum MDA levels by improving the antioxidant status, which directly reflects, reduction in the oxidative stress and lipid peroxidation in these subjects. This suggested an additional supplementation of oral antioxidant vitamins and minerals, to group I cases, may be utilized for defending and inactivating the preexisting free radicals and those generated during radiotherapy, to lower free radical action. The reduced LPO and improved antioxidant system during radiotherapy in Group I patients may be helpful in reducing free radical damage to the normal tissues than malignant tissue thereby acting as radio protective for normal cells, which may decrease the chances of developing secondary tumors.

However the group II head and neck malignancy patients not supplemented with oral antioxidant tablets showed increased levels of serum MDA and decreased levels of serum TAS, which indicates that the mode of radiotherapy has generated

additional free radicals during the irradiation process, and less defended by poor antioxidant system in these individuals. The high production of free radicals during radiotherapy may be utilized for killing and shrinking the tumor cells to produce a good clinical response in treated cases. The generated free radicals through γ (gamma) radiations during radiotherapy and not effectively inactivated by antioxidant system may further increase the chances of secondary tumors in the group II treated malignant cases.

An earlier case control study demonstrated a marked LPO in malignancy patients irradiated at different anatomic fields (14). A good correlation of lipid peroxidation and alpha-tocopherol supplementation in patients with cervical carcinoma, receiving radical radiotherapy was found to be effective and significant, whose results were in concordance with the present study results (15).

A recent study reported, supplementation with high doses of alpha-tocopherol and beta-carotene during radiation therapy could reduce the severity of disease and antioxidant supplementation as an adjuvant therapy, which might compromise the radiation treatment efficacy (16, 17). Efficacy of vitamin A supplementation in prevention of loco-regional occurrence and second primaries in head and neck cancer was evidenced by Jyothirmayi et al (18).

The overall study findings concludes, that the estimations of serum MDA and serum TAS in head and neck malignancy cases before and after radiotherapy serves as a good indicators of oxidative stress in different stages, correlating with the severity and stage of head and neck malignancies. The study concludes that an oral antioxidant supplementation has effectively reduced LPO and oxidative stress due to increased antioxidant status in the individuals supplemented during radiotherapy. Antioxidant supplementation during radiotherapy may serve as an adjuvant therapy in malignancies offering a good protection to normal cells that may further reduce the risk of developing secondary cancers. However a larger patient cohort with a longer follow up period for therapeutic response studies may yield more significant data.

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