ORIGINAL ARTICLE

Biochemistry Section

The Role Of **Oxidative Stress** And The **Effect Of Radiotherapy** On The **Plasma Oxidant-Antioxidant** Status In **Head** And **Neck Cancer**

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ABSTRACT

Objectives: The present study was aimed to assess the oxidant-antioxidant status in head and neck cancer patients before and after radiotherapy.

Methods: The subjects comprised of patients who had visited the Oncology Department and were diagnosed of head and neck cancer. The plasma levels of malondialdehyde (MDA), the marker of lipid peroxidation, and the antioxidants, superoxide dismutase (SOD), vitamin A, vitamin C and ceruloplasmin, were assayed before and after radiotherapy, in comparison to the healthy controls.

Results: 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. These parameters showed significant changes after radiotherapy, as indicated by a lower level of MDA and higher levels of SOD, vitamin A, vitamin C and ceruloplasmin in the plasma of the cancer patients after radiotherapy, as compared to the plasma levels before radiotherapy. All the results were statistically significant ($P < 0.001$).

Conclusions: Radiotherapy caused a reduction in the lipid peroxidation and an improvement in the antioxidant status of the head and neck cancer patients.

Key Words: Antioxidants, Head and Neck Cancer, Lipid Peroxidation, Oxidative Stress

INTRODUCTION

Cancer is the second leading cause of death worldwide. Eleven million new cases of cancer are diagnosed every year [1]. It is estimated that there are approximately 2-2.5 million cases of cancer in India at any given point of time, with around 700,000 new cases being detected every year [2]. Cancers of the head and neck include cancers of the buccal cavity, the head and neck subset, the larynx, pharynx, thyroid, the salivary glands, and the nose/nasal passages. Head and neck cancers account for approximately 6 percent of all the malignancies in the United States, and over one third of all the cancers in India [3], [4]. It is the sixth common cause of death in males and the seventh in females [3]. India also has the dubious distinction of having the world's highest reported incidence of head and neck neoplasia in women. The disproportionately higher prevalence of head and neck cancers with respect to other malignancies in India may be due to the use of tobacco in various forms, the consumption of alcohol and a low socioeconomic condition which is related to poor hygiene, a poor diet or infections of viral origin [4].

Free radicals are implicated in the pathogenesis of a multistage process of carcinogenesis. They are proposed to cause DNA base alterations, strand breaks, damage to the tumour suppressor genes and an enhanced expression of the proto-oncogenes [5 -8]. The burst of the reactive oxygen species (ROS) and the reactive nitrogen species (RNS) has been implicated in the development of cancer. Increased levels of lipid hydroperoxide, MDA and nitric oxide and decreased levels of the antioxidants, catalase, SOD, glutathione peroxidase, vitamin C and vitamin E, in blood and tissues, have been reported in head and neck cancer patients [9-15].

Radiotherapy is a cornerstone in the treatment of head and neck cancer. Ionic irradiation exposes all cells in the involved field to high levels of oxidative stress, thus resulting in the formation of ROS, increasing DNA damage and ultimately leading to cell death [16]. Another mechanism of the action of radiotherapy is to alter cellular homeostasis, thus modifying the signal transduction pathways and predisposing to apoptosis [16]. However, there are conflicting reports on the effect of radiotherapy on oxidative stress. Some studies have reported increased oxidative stress after radiotherapy [17], [18] while others have reported decreased oxidative stress after radiotherapy in cancer patients [18], [19], [20]. These studies have shown varied responses of the individual antioxidants to radiotherapy.

The present study was taken up with a view of the paucity of Indian studies on the effects of radiotherapy on the oxidant-antioxidant status in cancer and the existing lacunae in the field of oxidative stress biomarkers of cancer. We assessed MDA, the marker of lipid peroxidation, and the antioxidants, SOD, vitamin A, vitamin C and ceruloplasmin, in the blood of head and neck cancer patients, before and after radiotherapy.

MATERIALS AND METHODS

Source of Data: The study was carried out at the Father Muller Medical College and Hospital, Mangalore. The study protocol was approved by the Institutional Ethics Committee. The subjects comprised of fifty patients who had visited the Oncology Department and were diagnosed of head and neck cancer, based on clinical examination and histopathological studies; they ranged in ages from 30 to 80 years (Group-1A). The same patients were then followed up after they underwent a radiotherapy regimen for six weeks (Group-1B). Fifty, age- and sex-matched, apparently healthy volunteers were included as controls in the study (Group-2). Patients with cancer in regions other than the head and neck, any

systemic diseases and infections, and alcoholics, smokers and tobacco chewers without diagnosed cancer, were excluded from the study. A detailed history of the habits (smoking, alcohol abuse and tobacco chewing), chronic illness and clinical history, was collected from the subjects. Informed consent was obtained from all the participants.

Assays: Six ml. of blood was collected in an EDTA tube by taking aseptic precautions, and this was centrifuged at 3000 rpm to separate the cells and plasma. The plasma was analyzed for the levels of MDA, vitamin A, vitamin C and ceruloplasmin, and the activity of SOD. MDA, the sensitive and convenient marker of lipid peroxidation, was estimated as thiobarbituric acid-reactive substances [21]. The plasma levels of vitamin A (retinol) were assayed after extraction into heptane and by then measuring the absorbance at 327 nm [22]. The measurement of vitamin C (ascorbic acid) was based on the oxidation of ascorbic acid to dehydroascorbic acid, which on treating with 2,4 dinitrophenyl hydrazine in an acidic medium, formed a red coloured bishydrazone [23]. The ceruloplasmin levels were estimated, based on the oxidation of p-phenylene diamine, the colour intensity of which was measured at 530 nm [24]. SOD was estimated as super oxide anions are involved in the auto oxidation of pyrogallol at an alkaline pH of 8.5. SOD inhibits the auto oxidation of pyrogallol, which can be determined as an increase in its absorbance at 420nm [25].

Statistical Analysis: The results were evaluated by employing the unpaired "t" test, the paired "t" test and the Chi- square test.

RESULTS

In a total of fifty patients with head and neck cancer, the major anatomical sites of the carcinoma were the tongue (n=13), the oropharynx (n=6) and the hypopharynx (n=4). The remaining sites with a lesser incidence were the buccal mucosa (n=3), the mandible (n=3), the cheek (n=3), the nasopharynx (n=3), the pyriform fossa (n=3), the maxilla (n=3) the vocalcord (n=3), and one each in the floor of the mouth, the supraglotis, the pharynx, the post cricoids, the retromandibular trigone and the right maxillary antrum. The results of the present study are presented in [Table/Fig 1].

	1. Head and Neck Cancer patients (n=50)		
	1A. Before radiotherapy	1B. After radiotherapy	2. Controls (n=50)
MDA (nM/dL)	1849.76±246.63 **	1513.16±269.94 ***	496.88±124.9
SOD (U/mL)*	3.10±0.68 **	4.16±0.54 ***	6.23±1.2
Vitamin C (mg/dL)	0.332±0.11 **	0.43±0.14 ***	1.042±0.2
Vitamin A (µg/dL)	25.70±8.61 **	30.88±6.36 ***	47.42±10.2
Ceruloplasmin (mg/dL)	16.48 ±3.75 **	22.30 ±4.74 ***	32.58±6.88

[Table/Fig 1]: Plasma Oxidant-Antioxidant Status in Head and Neck Cancer (Values are mean ± S.D. of number of subjects indicated)

* One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation.
** Significance of difference of the value when compared to controls, P < 0.001 (Highly significant)
*** Significance of difference of the value when compared to before radiotherapy, P < 0.001 (Highly significant)

The plasma levels of malondialdehyde were higher and the levels of superoxide dismutase, vitamin A, vitamin C and ceruloplasmin were lower in the head and neck cancer patients (Group-1A.), as compared to those in the controls (Group-2). These parameters showed significant changes after radiotherapy, as indicated by lower levels of MDA and higher levels of SOD, vitamin A, vitamin C and ceruloplasmin in the plasma of cancer patients after radiotherapy (Group-1B), as compared to the plasma levels before radiotherapy (Group-1A). All the results were statistically significant (P < 0.001).

DISCUSSION

The present study revealed increased lipid peroxidation and lowered levels of antioxidants in the head and neck cancer patients. The levels of MDA, the marker of lipid peroxidation, were higher almost by 4-fold in the cancer patients as compared to the controls. The levels of the antioxidants, SOD, vitamin C, vitamin A and ceruloplasmin, were decreased in the head and neck cancer patients. This suggests an increased oxidative stress being involved in the pathogenesis of head and neck cancer.

Previous studies have reported increased blood levels of lipid peroxides and MDA, and decreased blood levels of antioxidants in head and neck cancers [8-15]. Red blood cell membranes are more prone to lipid peroxidation because of their high polyunsaturated fatty acid content and their direct exposure to molecular oxygen and haemoglobin. The compensatory mechanism to counter the ROS results in reduced levels or activities of enzymatic and non-enzymatic antioxidants in blood.

Radiotherapy is the major form of treatment which is available for head and neck cancer. Studies have shown varied findings with respect to the effect of radiotherapy on the oxidant-antioxidant status. Some authors have observed increased oxidative stress after radiotherapy and have suggested that radiation-induced free radicals cause oxidative damage to biomolecules [17], [18], [26]. The authors advocating increased oxidative stress in radiotherapy have shown from their studies, that supplementation with antioxidants such as alpha-tocopherol ameliorates the oxidative damage caused by radiotherapy [26]. Chitra and Shyamaladevi [26] observed a significant decrease in the malondialdehyde levels and an increase in the activities of antioxidant enzymes in oral cancer patients who were supplemented with alpha-tocopherol during radiotherapy, as compared to radiation-treated oral cancer patients without alpha-tocopherol supplementation.

Studies have shown decreased lipid peroxidation and increased activities of antioxidants after radiotherapy [18], [19], [20]. The findings from our study also suggest the amelioration of oxidative stress by radiotherapy, as indicated by decreased MDA levels, and increased levels of SOD, vitamin C, vitamin A and ceruloplasmin in head and neck cancer patients after radiotherapy. Kasapovic et al. [18] proposed that the response to radiotherapy involves the age-related impairment of the antioxidant capacity for the elimination of free radicals, thus causing oxidative damage to the blood cells. They suggested that the cytotoxic effects of radiation on the healthy tissues might be more pronounced during the aging process. In concordance with our study, some researchers have observed increased blood levels of SOD, catalase, glutathione reductase, ceruloplasmin and glutathione in post-radiotherapy cancer patients [18], [19], [20].

CONCLUSIONS

The involvement of oxidative stress in head and neck cancer is evident from increased lipid peroxidation and decreased levels of antioxidants in plasma. Radiotherapy caused a reduction in lipid peroxidation and an improvement in the antioxidant status of head and neck cancer patients. Though the levels of antioxidants in plasma did not reach the control values in the post-radiotherapy scenario in cancer patients, the improvement in the antioxidant capacity was of vital importance for survival and for an improved quality of life of

the radiation-treated patients. Further studies with a larger sample size, different stages of head and neck cancer and assessment of the effect of antioxidant supplementation before and during the radiotherapy regimen, are needed.

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