Alteration of Lipid Peroxidation and Total Antioxidant Capacity in Patients With Head and Neck Cancers Following Radiotherapy

Soheila Manafizadeh, Farid Abbassi, Reza Mirzaii Dizgha, Roya Khatami, Mostafa Esmseif, and Afshin Almasi

Abstract

Background: Head and neck cancers are one of the main causes of cancer-related death worldwide. The common approach in the treatment of head and neck cancers is radiotherapy either alone or in combination with additional therapeutic strategies. The aim of this study was to evaluate the effects of radiotherapy on lipoperoxidation and total antioxidant levels in patients with head and neck cancers.

Methods: Lipid and total antioxidant levels were determined in patients with head and neck cancers before radiotherapy, 6 months after radiotherapy, and 6 months after chemotherapy. The levels of antioxidant capacity were significantly increased in patients receiving chemotherapy compared to patients receiving radiotherapy. Moreover, lipoperoxidation levels were significantly decreased in patients receiving chemotherapy compared to patients receiving radiotherapy. In addition, lipoperoxidation levels slightly increased in patients receiving radiotherapy and chemotherapy compared to patients receiving chemotherapy alone.

Results: Antioxidants, lipids and total antioxidant levels, were significantly increased in patients receiving chemotherapy compared to patients receiving radiotherapy. However, antioxidant levels dropped to normal levels in patients receiving radiotherapy alone.

Conclusion: Radiotherapy is a common approach in the treatment of head and neck cancers. Radiotherapy induces DNA damage in cancer cells, which can lead to increased levels of ROS. In addition, radiotherapy causes DNA damage, genomic instability, and tumor suppressor genes reduction as well as increased expression of pro-inflammatory genes. Increased levels of lipid peroxidation, shown by malondialdehyde (MDA), have been reported in different types of cancer. Conversely, the levels of total antioxidant capacity and related enzymes such as glutathione peroxidase, superoxide dismutase, and catalase decrease in patients with head and neck cancers.

1. Background

Head and neck cancers are one of the most common causes of cancer-related death worldwide (1). Head and neck cancers are heterogeneous groups of tumors originating from different anatomic sites. The prevalence of head and neck cancer is related to diverse risk factors including age, sex, and geography. In addition, head and neck cancers are predominantly associated with smoking and alcohol drinking (2). Free radicals as inevitably produced in metabolic processes in normal cells play important roles in cancer pathogenesis. Elevated reactive oxygen species (ROS) induces DNA damage, genomic instability, and tumor suppressor genes reduction as well as increased expression of pro-inflammatory genes (3). Increased levels of lipid peroxidation, shown by malondialdehyde (MDA), have been reported in different types of cancer. Conversely, the levels of total antioxidant capacity and related enzymes such as glutathione peroxidase, superoxide dismutase, and catalase decrease in patients with head and neck cancers (5, 6).
matrix have compelling reasons behind. In this regard, it clearly offers an inexpensive, non-invasive and easy-to-use screening method. In addition, it has several advantages over serum and urine for collection, storage, shipping and volumetric sampling. Moreover, handling of oral fluid during csiratory procedures is far easier than blood, because it does not clot, thus reducing the number of required manipulations. Furthermore, noninvasive nature of saliva collection approach could dramatically reduce a patient's willingness to continue health-related examinations over time (14,16).

2. Objectives

Here, the objective of the present study was to evaluate oxidative stress status in patients with head and neck cancers before and after radiotherapy using measurements of lipid peroxidation and total antioxidant capacity levels.

3. Patients and Methods

3.1. Patients and Clinical Conditions

Whole intact saliva samples were obtained from 47 patients with head and neck cancers referred to Imam Khomeini Hospital (Tehran, Iran) for radiotherapy from January to September 2016, comprising 33 males and 14 females in pre- and post-radiation. Seventeen patients were excluded because of ageostomia and finally 9 males and 27 females were analyzed in both pre- and post-radiation. The mean age of patients was 66.9 years (ranged 55-85 years) and they were compared with a control group of 30 healthy individuals including 16 females and 14 males with a mean age of 27.6 years without any history of smoking and alcohol consumption. The conditions of all patients concerning antibiotic prophylaxis, nutrition and preoperative treatment were controlled. In this study, unstimulated saliva was used and saliva induction was prevented by avoiding patients and healthy controls for drinking, eating, smoking and all kinds of actions that may induce saliva secretions two hours before samples collection. Collected samples were kept at -70°C until use.

3.2. Determination of Lipid Peroxidation Using Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC)

TAC of saliva was determined by measuring its ability to decolorization of AIBS radical cation according to previous fully described methods and the assay calibrated with TROLOX (17).

Saliva MDA levels were determined by a method based on reaction with thiobarbituric acid (TBA) at 90-100°C (18). MDA and TBA react together in the TBA test reaction to produce a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 90°C for 15 minutes. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was palliated by centrifugation and an aliquot of supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 30 min. After cooling, the absorbance was read at 532 nm. The results were expressed as pmol/ml, according to a standard curve, which was prepared with a serial dilution of standard 1,1,3,3-tetramethoxypropane.

3.3. Statistical Analysis

Differences of lipid peroxidation using Malondialdehyde (MDA) and total antioxidant capacity levels in saliva of patients and healthy control in various groups including pre-radiotherapy and post-radiotherapy and comparison of these levels with control healthy group were analyzed by ANOVA and Tukey's as Post hoc using SPSS software version 16.00 (SPSS Inc., Chicago, IL, USA).

4. Results

4.1. Levels of Total Antioxidant Capacity

One way ANOVA indicated that saliva total antioxidant capacity was altered by radiotherapy (F (2, 87) = 20.635; P = 0.001) (Figure 1). Post-hoc analysis showed that TAC was significantly lower in head and neck cancer group before radiotherapy than after radiotherapy and control group, interestingly, the level of TAC was remarkably enhanced following radiotherapy in patients compared with before radiotherapy (P < 0.05).

![Figure 1](image)

Data are expressed as mean ± SEM. *Different from control; # different from after radiotherapy, P < 0.05.)
4.2. Levels of Lipid Peroxidation (MDA)

There was no significant difference in the lipid peroxidation as expressed by MDA levels among groups ([7, 87] = 1.013; P = 0.315) (Figure 2).

Figure 2. Unstimulated white blood cell concentration of malondialdehyde in patients with head and neck cancer before and after radiotherapy and control individuals.

Data are expressed as mean ± SEM.

5. Discussion

In the current study, total antioxidant capacity was low in patients with head and neck cancer before radiotherapy. After radiotherapy, the levels of total antioxidant capacity increased. Furthermore, lipid peroxidation was slightly high in patients with head and neck cancers, but the levels of MDA as marker of lipid peroxidation decreased following radiotherapy.

The oxidative stress induced lipid peroxidation to generate wide range of products including MDA. Previous investigations showed that MDA plays an important role in carcinogenesis through interaction with DNA and formation of DNA-MDA adducts. DNA-MDA adducts induces mutations in various genes such as tumor suppressor genes and oncogenes as well as cell cycle alterations in several cancers ([6]).

Increased levels of lipid peroxidation alongside decreased levels of antioxidant capacity have been severally reported in patients with head and neck cancers ([5, 20]). Increased levels of lipid peroxidation and MDA are largely associated to damage red blood cell membranes enriched by polyunsaturated fatty acids. The levels of free radicals increase in head and neck cancers leading to enhanced lipid peroxidation. On the other hand, the levels of antioxidant capacity decreased following enhancement of ROS production in compensatory manner ([21]).

Free radicals, as mentioned above, play crucial roles in oxidative stress. Some studies showed that the levels of ROS products increased by radiotherapy resulting in enhancement of oxidative stress ([22, 23]). Furthermore, some authors indicated that radiotherapy-mediated oxidative stress decreased using antioxidant such as alpha tocopherol ([24]). Conversely, some other studies indicated that lipid peroxidation decreased after radiotherapy alongside improvement of antioxidant capacity status ([21, 25]). Decreased levels of MDA may be related to death of tumor cells and tumor load decline as the main source of ROS.

In conclusion, lipid peroxidation slightly reduced in patients with head and neck cancers after radiotherapy. However, antioxidant status seems to be improved in these patients after radiotherapy.

Acknowledgments

We would like to thank all patients and other volunteers for their contribution to this study.

Footnote

Authors’ Contributions

All authors contributed in the analysis and interpretation of data, drafting the manuscript and revising it, and gave final approval of the version to be published.

References
