Effect of Antioxidants on Markers of Apoptosis in Post-operative Radiotherapy of Cancer Cervix

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Aim of the work

To investigate whether a mixture of antioxidant supplementation can ameliorate damaging effects of ionizing radiation in cancer cervix patients during radiotherapy Accordingly apoptosis, lipid peroxides and Fas among cancer cervix patients undergoing post-operative radiotherapy (n=40) were measured before and after administration of a mixture of antioxidants including 60 mg vitamin C, 10 mg vitamin E, 1000 IU vitamin A and 50 mg Selenium.

Patients & Methods

Patients were divided into 2 groups each of 20 patients. Antioxidant mixture was administered to one group thrice per day during the duration of radiotherapy and one more week post cessation of radiotherapy. Twenty normal healthy women participated as controls.

Results

Results revealed that following the first and second sessions of radiotherapy, both the groups with and without antioxidant administration showed higher frequency of lipid peroxidation and the frequency of micronuclei compared to their level before radiotherapy as measured 24 hours and 48 hours post first session of radiotherapy. Patients undergoing radiotherapy showed a decrease in all parameters of the study after one-week, one-month post irradiation and one week post cessation of radiotherapy. However, the antioxidants group showed significantly lower levels of apoptosis and lipid peroxides and Fas compared to patients who did not receive antioxidant supplementation.

Conclusion

The results of the present study reveals adaptation to radiation during radiotherapy and evaluate the prophylactic effect of a mixture of antioxidants during radiotherapy.

Key words

Antioxidant, apoptosis, radiotherapy, cancer cervix.

Introduction

As an integral part of cancer-conserving treatment, radiotherapy reduces the incidence of local and regional recurrences. Ionizing radiation can damage cells directly by interacting with critical cellular targets or indirectly by generating free radicals [1]. Regardless of the mechanism, radiation-induced damage often triggers the endogenous suicide machinery of cells [2]. Inflammation is a frequent radiation-induced reaction following therapeutic irradiation [3]. The primary response of inflammation is mediated by a series of inflammatory cascades, which result from a complex interplay between a variety of immune and non-immune cells including endothelial smooth cells, macrophages and polymorphonuclear leucocytes [4]. The primary step involves the recruitment of PMNs and the subsequent release of reactive oxygen species (ROS) [5] and reactive nitrogen species (NOS) [6]. In this aspect, ionizing radiation has been well established to stimulate the release of ROS.

Under normal conditions ROS are scavanged by an endogenous efficient mechanism including enzymes, metabolites, molecules and dietary vitamins [7]. However, during diseases states and exposures to external environmental stimuli

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such as ionizing radiation the generation of ROS exceeds the capacity of endogenous antioxidant defenses leading to a disturbance of the oxidant/antioxidant balance in favour of the former [8]. ROS attack macromolecules including protein, DNA and lipid etc. causing cellular/tissue damage. To counter their effect, the diseased body can be supplemented from exogenous sources, minerals like Se, and vitamins like vitamin A, C and E [9].

The aim of this present study was to investigate whether supplementation of vitamin A, E, C and selenium which are potent antioxidants could ameliorate the damaging effects of ROS on DNA during radiotherapy measured in terms of apoptosis, DNA-fragmentation, DNA-protein-cross link and the frequency of micronuclei.

Subjects and Methods

Patients:

Forty patients suffering from cancer cervix, whose ages ranged from 42- 60 years with a mean of (48.5 ± 8). Post-operative radiation at a dose of 200 rads daily was given for 4-5 weeks only. Blood samples were drawn just before treatment and then 24 hours, 48 hours, one-week, one-month post irradiation and one-week post cessation of radiotherapy. The plasma was assayed for MDA and protein carbonyls. Blood lymphocytes were assayed for MN, apoptosis, DNA fragmentation and results were compared with that of 20 controls with a mean age of 52 ± 6.

Radiotherapy

Patients were exposed to daily doses of 200 rads as a postoperative adjuvant treatment for cancer cervix.

Antioxidant Adminstration

Patients were divided into two groups, one of which received daily doses of a mixture of antioxidants including 60 mg vitamin C, 10 mg vitamin E, 1000 Ul vitamin A and 50 mg Selenium. The other group did not receive any vitamin supplementation.

Apoptosis

Lymphocytes were subjected to Histopaque separation, Fixed with 70% ethanol for 1 hour

and inspected by the fluorescent microscope after being incubated fluorscein diacetate for 15 minutes at 37°C and then incubated at 37°C with 10 μg RNA-ase and stained with 10-μg propidium iodide[10]. And inspected under the fluorescent microscope. This is a double staining technique, where viable cells fluorescent green and apoptoic cells fluorescence red.

Determination of Soluble Fas was duplicate in duplicate plasma samples. Soluble Fas protein was measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) [11].

Determination of Plasma Malondialdehyde

Lipid peroxidation was measured flourometrically in terms of plasma malondialdehyde [12].

Results

Apoptosis

Apoptosis in peripheral blood circulating lymphocytes showed a significant increase in apoptotic lymphocytes among total cancer cervix patients before radiotherapy when compared to controls (mean ± SD = 14.5 % ± 3.3 vs. 2 % ±. 0.02, p <0.001). Meanwhile, an increase in percentage of apoptotic cells after 24 and 48 hours can be observed in both groups, which decreased when measured after one week, one month and one week post cessation of radiotherapy in both groups compared to its level in each group (Figure 4). Vitamin supplementation reduced percentage of apoptotic cells in cancer cervix patients supplemented with vitamins compared to those without radiotherapy after 24 hours (17.8 ± 2.2 % vs. 19.6 ± 2.3 %), 48 hours (21.5 ± 2.3 % vs. 25.6 ± 3 %), one week (19.5 ± 3.2 % vs. 22.7 ± 4.2 %), three weeks (15 ± 2.1 vs. 19 ± 1.6) and one-week post cessation of radiotherapy (10 ±. 1.5 % vs. 14 ± 1.8 %).

Lipid Peroxidation:

Plasma malondialdehyde showed a significant increase in lipid peroxidation measured in terms of plasma malondialdehyde among total cancer cervix patients before radiotherapy when compared to controls (mean ± SD = 8.1 % ± 1.6 vs. 3.8 ±. 1.5 nmole/ml Meanwhile, an increase

in the level of plasma malondialdehyde after 24 and 48 hours can be observed in both groups, which decreased when measured after one week, three weeks and one week post cessation of radiotherapy in both groups compared to its level in each group (Figure 4). Vitamin supplementation reduced percentage of apoptotic cells in cancer cervix patients supplemented with vitamins compared to those without radiotherapy after 24 hours (7.8 ± 1.2 % vs. 9.6 ± 2.5 nmole/ml), 48 hours (11.5 ± 2.3 % vs. 15.6 ± 3), one week (9.5 ± 2.6 % vs. 12.7 ± 3.2 nmole/ml), three weeks (5 ± 1.6 vs. 9 ± 2.8 nmole/ml) and
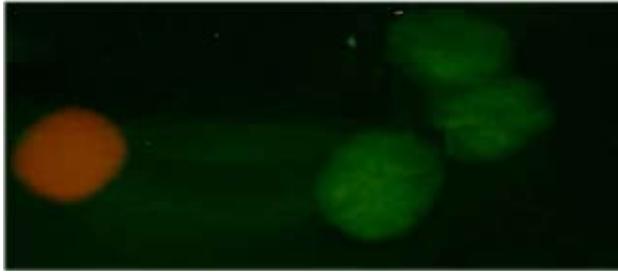

Fig. 1 : Apoptotic lymphocytes cell among cancer cervix patients undergoing RT with and without antioxidants
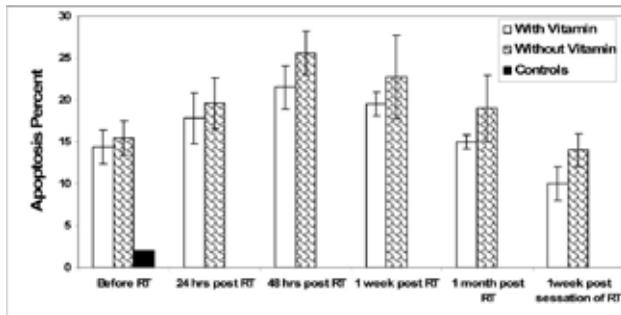

Fig. 2 : Percent of apoptotic lymphocytes cell among cancer cervix patients undergoing radiotherapy with and without antioxidant intake: before and after 24 hrs, 48 hrs, one week. 3 weeks and 1week post cessation of radiotherapy one month.
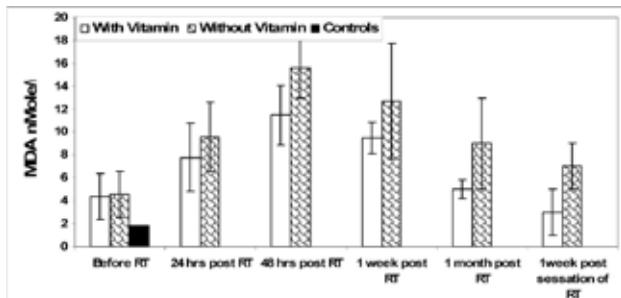

Fig. 3 : Level of plasma malondialdehyde among cancer cervix patients undergoing radiotherapy with and without antioxidant intake before and after 24 hrs, 48 hrs, one week., one month and 1 week post cessation of radiotherapy one month
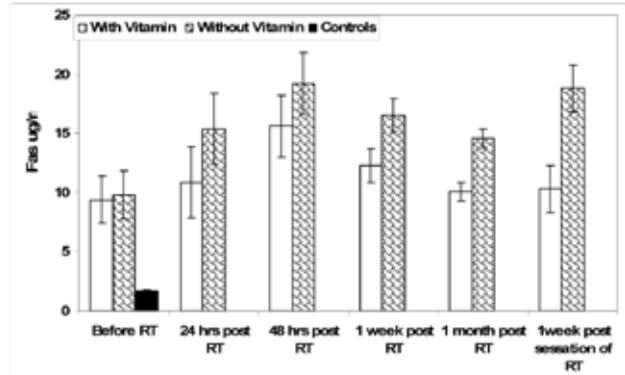

Fig 4 : Level of plasma Fas among cancer cervix patients undergoing radiotherapy with and without antioxidant intake before and after 24 hrs, 48 hrs, one week, one month and 1 week post cessation of radiotherapy one month

one-week post cessation of radiotherapy. (3 ±. 1.5 vs. 7 ± 2.5 nmole/ml).

Fas

Fas in peripheral blood circulating lymphocytes showed a significant increase Fass among total cancer cervix patients before radiotherapy when compared to controls (mean ± SD = 9.8 ± 1.7 vs. 1.9 ±. 0.02, $p < 0.001$). Meanwhile, an increase in Fas after 24 and 48 hours can be observed in both groups, which decreased when measured after one week, three weeks and one week post cessation of radiotherapy in both groups compared to its level in each group (figure 5). Vitamin supplementation reduced fas in cancer cervix patients supplemented with vitamins compared to those without radiotherapy after 24 hours (10.9 ± 2.1 vs. 15.4 ± 1.3), 48 hours (15.6 ± 3 vs. 19.2 ± 5), one week (10.2 ± 4.3 % vs. 16.5 ± 4.9), three weeks (10.1 ± 4.3 vs.14.6 ± 5) and one-week post cessation of radiotherapy. (10 ± 3.2 vs. 18.8 ± 2.4).

Discussion

Radiation therapy is one of the major treatment modalities in the management of human cancer. While impressive progress like more accurate dosimetry and more precise methods of radiation targeting to tumor tissue has been made, the value of radiation therapy in tumor control may have reached a plateau. At present, two opposing hypotheses regarding the use of antioxidants during radiation therapy have been proposed. One hypothesis states that supplementation with high doses of multiple micronutrients including

high dose dietary antioxidants (vitamins C and E, and carotenoids) may improve the efficacy of radiation therapy by increasing tumor response and decreasing some of its toxicity on normal cells [13]. Vitamin A has been shown to reduce irradiation-induced fibrosis after irradiation [14]. The other hypothesis suggests that antioxidants (dietary or endogenously made) should not be used during radiation therapy, because they would protect cancer cells against radiation damage [15].

The abrupt increase is a consequence to the radiation dose, which the patients received. The gradual decrease can be explained by an adaptive response, which the body normally under goes in order to protect itself against ionizing radiation and other environmental stresses. It has been long established that when organisms or cells are exposed to low levels of specific harmful physical or chemical agents, a beneficial physiologic effect is observed. Concurrently; exposure to sub-lethal challenges of stress may rejuvenate the cell by repairing damage before the challenge and may provide transient protection against further damage from subsequent sub lethal or lethal challenges with a different otherwise harmful physical or chemical stressor [16,17]. It has been suggested that a mechanism contributing to these beneficial effects of otherwise harmful agents may operate through the stress response (SR) 18. A physiologic mechanism characterized by the induction of families of detoxification and repair molecules [19,20], induction of DNA repair and antioxidant enzymes [9] and programmed cell death [21]. The SR has been characterized using heat (the classical SR), radiation, heavy metals, and oxidizing agents [10].

Apoptosis or programmed cell death is considered as a stress response mechanism [22], which is characterized by certain morphological features such as loss of membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and intranucleosomal cleavage of DNA. The dying cells fragmenting into apoptotic bodies, which are rapidly eliminated by phagocytic cells without eliciting significant inflammatory damage [23]. The homeostatic balance between cell proliferation and apoptosis

in the maintenance of constant cell numbers may provide a hormetic effect by minimizing the consequences of proliferation-related mutagenesis during tumour promotion [24]. The adaptive increase in apoptosis that accompanies the oncogene-activated deregulation in proliferation selectively eliminates potentially preneoplastic cells in hyperplastic foci. Apoptosis is considered as one of the antioxidant mechanisms for the elimination of oxidative damage to cellular DNA [9]. In this present study it is clear that vitamins decreased the percentage of apoptotic cells in circulating lymphocytes of those patients who were undergoing radiotherapy indicating the efficiency of the given antioxidants in protection against DNA damage and a decrease of micronuclei.

The abrupt increase in plasma MDA followed by a gradual decrease could be explained in terms that the lipids in the path of the radiotherapy path is worn up and that an adaptive response has emerged and helped in decreasing MDA. It is evident that antioxidants reduces the intensity of MDA formation and thus efficient in decreasing the level of MDA, which is known to be a potentially important contributor to DNA damage and mutation that is produced endogenously via lipid peroxidation and prostaglandin biosynthesis. MDA is mutagenic in bacterial and mammalian cell assays, and it is carcinogenic in rats and to human cells [25].

In general Cancer patients undergoing radiotherapy they have low levels of selenium. Also exposure to ionizing radiation cause a decrease in both vitamins C and E [26,27]. And vitamin C is important for catalytic cycling of vitamin E. Similar results are also shown with vitamin A. Thus it is evident that supplementing patients with anti oxidants will be of great value in reducing side effects observed during radiotherapy [28-30]. In this present study we observe that after one week of the commencement of radiotherapy there is a marked decrease in all markers of oxidative stress including lipid peroxidation, apoptosis and Fas.

Antioxidant administration to cancer cervix patients undergoing post operative radiotherapy may ameliorate damaging effects of ionizing radiation.

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