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COMPARATIVE EFFECTS OF VARIOUS ANTIOXIDANTS ON THE HEMATOLOGICAL CHANGES DURING RADIOTHERAPY OF IRAQI FEMALES WITH BREAST CANCER

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ABSTRACT

Background. Radiation therapy involves production of reactive oxygen species (ROS) and induces oxidative stress condition, which attributed to the impairment of antioxidant defense mechanisms in the body especially in the blood. This study was designed to evaluate the mematoprotective effects of different antioxidants during radiation therapy in women with breast cancer. **Methods**. Seventy-five women with breast cancer and 20 healthy controls were included in the study. Hematological parameters (hemoglobin levels, white blood cell counts, and platelets counts) were measured pre- and post-radiotherapy for 4 weeks with and without antioxidant drugs. **Results**. The results indicated that in women with breast cancer, Hb, WBCs counts and platelets levels decreases lower than that of normal controls, and radiation therapy further decreases these parameters. **Conclusion**. The use of antioxidant drugs may have some protective effects against damaging effects of radiation therapy. Aspirin and allopurinol have some protective effects, but the radioprotective effect of melatonin was superior to the others.

Keywords: Radiotherapy, breast cancer, antioxidants, hematopoietic system.

INTRODUCTION

The use of radiation therapy as a central part of curative treatment for several types of cancer has been developed during the last decades [1]. Normal tissues neighboring to the tumor are going to receive variable quantities of radiation, which may result in damaging of these tissues and consequently emergence of adverse effects [2]. The severity of these adverse effects depend on many factors including the dose of radiation, rate of delivery, duration of treatment, type of radiation, site of exposure and the age of patient [3]. Ionizing radiation is considered as a powerful inducer of oxidative stress [4]. Radiation may induce oxidative damage both directly and indirectly, but the indirect pathway is much more important, because radiated water molecules, which are highly available in the body, resulted in a highly reactive and damaging chemical entities, leading to various types of harmful effects to the biomolecules especially the blood components [5]. Many clinical studies have reported modest decreases in treatment-related side effects when supplemental antioxidants (dietary or pharmaceutical) are administered concurrently with treatment regimens that include radiation and/or cytotoxic agents [6-8]. However, concern has been expressed that the action of supplemental antioxidants might not be restricted to reducing the oxidative damage to normal tissues generated by radiation therapy and certain chemotherapeutic agents [9,10]. Antioxidant supplementation during radiation therapy poses a conundrum for the radiation oncologist, as antioxidants that protect normal cells from reactive oxygen species may provide the same benefits to cancer cells and reduce the efficacy of treatment. Theoretically, antioxidants can exert their effects on all tissues to some degree, thereby protecting tumor cells as well as healthy ones. Experimental and clinical studies [11,12] supported this hypothesis, with some clinical data also suggesting that cancer patients who use antioxidant supplements during radiation and/or chemotherapy have worse survival than those who do not [13,14]. However, short- and long-term injury to healthy cells, including tissue damage and increased risk of oncogenic transformation can be prevented by antioxidants [15]. The present study was designed to evaluate the protective effect of different antioxidants against the radiation-induced hematological changes in Iraqi women with breast cancer.

PATIENTS AND METHODS

This study was carried out on 75 Iraqi females with breast cancer; they have age range of 35-55 years. After mastectomy and initial treatment firstly with systemic chemotherapy, they were exposed to local radiotherapy of breast and chest wall. The local Research Ethics Committee approved the research protocol and all patients signed a written consent before participation in the study. Radiation dose was prescribed (30-40 GY) in 20 fractions given in four weeks using three and four–field technique at the Iraqi Hospital of Radiology and Nuclear Medicine. Before initiation of radiotherapy, the patients were allocated into five groups (15 in each): group A, patients who did not receive any antioxidant drugs during radiation therapy. Meanwhile, the other groups were treated with different antioxidants for 4 weeks during radiotherapy, as follow: group B, patients treated with a combination of vitamin C (500 mg/day) and vitamin E (100 mg/day); group C, patients treated melatonin (3 mg/day) given at bed time; group D, patients treated with 100 mg aspirin daily, and group E, patients treated with allopurinol 100 mg daily. Additionally, 20 healthy females, with matched age and body weight, were included and served as non-radiated control group. Before starting radiotherapy, 10 ml of venous blood were collected from all subjects, (base line samples) and at the end of treatment. The collected blood samples were placed into heparinized tubes. Hemoglobin (Hb) levels were estimated according to the method of Drapkin and Austin (1935) [16]. White blood cells (WBC) and platelets counts were determined using automated analyzer (MS9 Melets & Schloesing laboratories).

Statistical analysis

The results were expressed as mean±S.E. GraphPad prism 5.0 software was utilized for data analysis. Paired and unpaired Students' *t*-test were used to compare with control and pre- and post-treatment values. ANOVA and Bonferroni's *post hoc* analysis were used to examine the differences among post-treatment values of different drugs. P value less than 0.05 was considered significant.

RESULTS

In table 1, before randomization for radiotherapy and antioxidant treatment, the selected patients demonstrated significant decrease in hemoglobin levels and WBC count, compared with normal subjects. However, platelets count was not significantly affected. After randomization to different treatment groups and initiation of radiotherapy, figure 1 clearly showed that when no antioxidants used during radiotherapy (group A), Hb levels were significantly decreased compared to both baseline and control values. However in groups treated with antioxidants, although Hb levels in all patients were not significantly different compared with controls, aspirin-treated group showed significant elevation in Hb levels after one month compared with baseline values, but still not significantly different compared with that in control subjects ($P>0.05$). Moreover, Hb levels in vitamins and melatonin treated groups showed non-significant decrease compared with pre-treatment values, but they were significantly lower than that in control subjects. Using ANOVA to compare the effects of different antioxidants, no significant differences among them were reported after one moth of treatment ($P>0.05$). In figure 2, when no antioxidants used during radiotherapy (group A), WBC count values were significantly decreased compared to both baseline and control values. Meanwhile, treatment with melatonin or allopurinol prevent the radiation-induced decrease in WBC count, and the values were not significantly changed compared with baseline values, but these levels were significantly lower ($P<0.05$) than that reported in control groups. However, in vitamins and aspirin treated groups, WBC counts were significantly decreased during radiotherapy, compared to baseline and control subjects values ($P<0.05$) after one month. Using ANOVA to compare the effects of different antioxidants on WBC count during radiation, no significant differences among them were reported after one moth of treatment ($P>0.05$). In figure 3, when no antioxidants used during radiotherapy (group A), Hb levels were significantly decreased compared to both baseline and control values. However, treatment with melatonin or aspirin prevent the radiation-induced decrease in platelets count, and the values were not significantly changed compared with baseline values, but these levels were still significantly lower ($P<0.05$) than that reported in control groups. Meanwhile, in vitamins and allopurinol treated groups, platelets counts were significantly decreased during radiotherapy, compared to baseline and control subjects values ($P<0.05$) after one month. Using ANOVA to compare the effects of different antioxidants on WBC count during radiation, no significant differences among them were reported after one moth of treatment ($P>0.05$).

Table 1: Hematological markers of breast cancer patients before initiation of radiotherapy

Parameters	**Control *n*= 20**	**Cancer patients Before radiotherapy *n*= 75**
Hemoglobin (gm/dl)	11.5±0.09	11.1±0.12*
WBC count (10^9/L)	6.0±0.18	4.8±0.12*
Platelet count (10^9/L)	195.5±2.95	190.5±3.01

Each value represents mean ± S.E.; *n*= number of subjects;* significantly different compared with control (p<0.05)

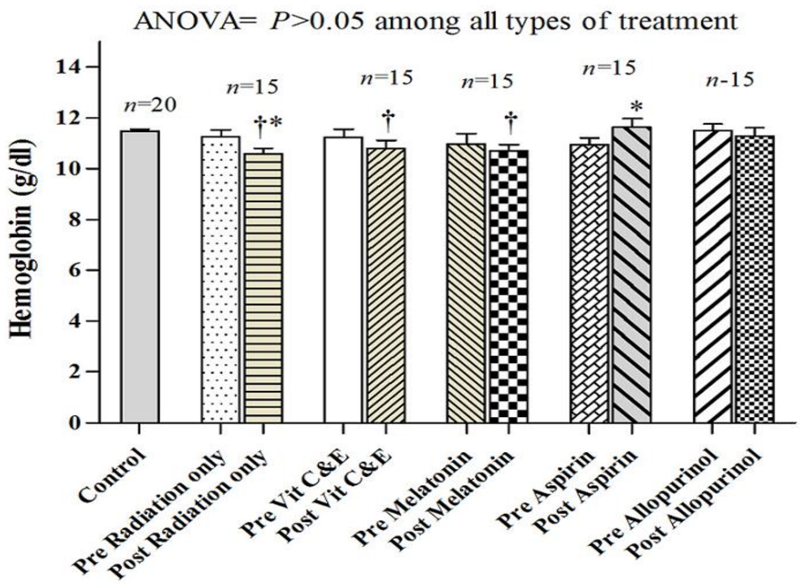

Figure 1. Effects of treatment with vitamins (C and E), melatonin, aspirin, and allopurinol on Hb levels of breast cancer patients maintained on radiation therapy. Each value represents mean±S.E.; *n*= number of subjects; † significantly different compared to control (*P*<0.05); * significantly different compared with baseline values (*P*<0.05).

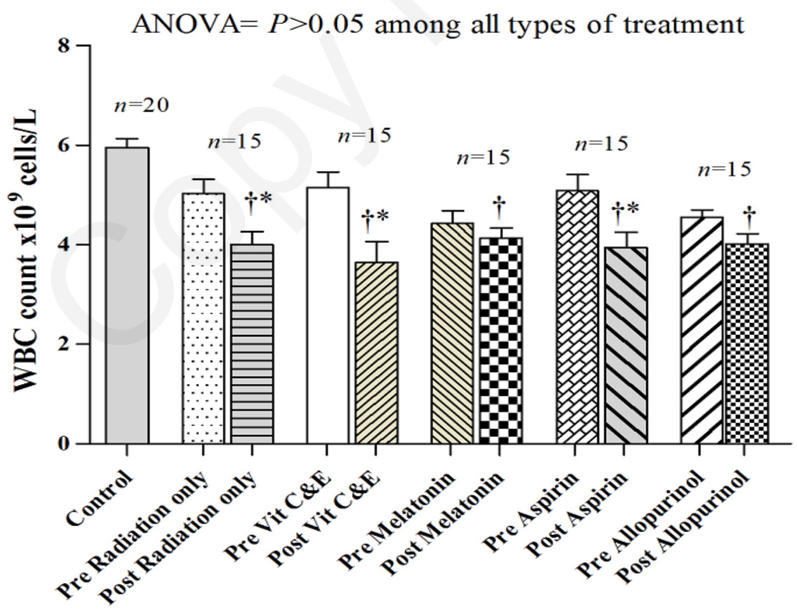

Figure 2. Effects of treatment with vitamins (C and E), melatonin, aspirin, and allopurinol on WBC count of breast cancer patients maintained on radiation therapy. Each value represents mean±S.E.; *n*= number of subjects; † significantly different compared to control (*P*<0.05); * significantly different compared with baseline values (*P*<0.05).

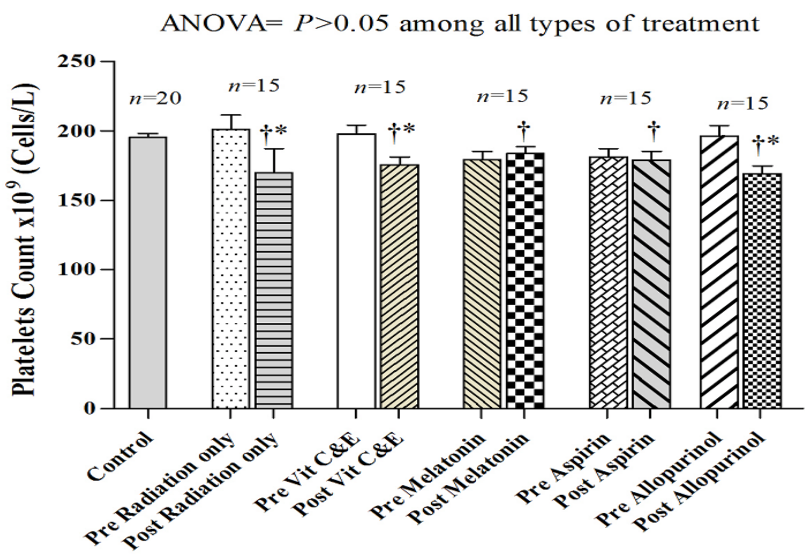

Figure 3. Effects of treatment with vitamins (C and E), melatonin, aspirin, and allopurinol on platelets count of breast cancer patients maintained on radiation therapy. Each value represents mean±S.E.; *n*= number of subjects; † significantly different compared to control (*P*<0.05); * significantly different compared with baseline values (*P*<0.05).

DISCUSSION

Radiation therapy initiates ionization leading to free radicals formation, which affects the antioxidant defense mechanisms of the body, aggravating the already present state of oxidative stress induced by cancer pathophysiology [17]. In the course of treatment, radiation produces numerous biological perturbations in cells; because normal cell toxicity limits the doses used in effective treatment, approaches are designed to strike a balance between eliminating cancer cells and protecting normal tissues. Radiation damages cells by direct ionization of DNA and other cellular targets and by indirect effect through (ROS). Exposure to ionizing radiation produces oxygen-derived free radicals in the tissue environment; these include hydroxyl radicals (the most damaging), superoxide anion radicals and other oxidants such as hydrogen peroxide [15]. Despite the substantial limitations of other studies [18,13], it is troubling that the reported results suggesting poorer survival with concurrent administration of antioxidants and cytotoxic therapy, even though these results are at odds with other studies. For example, two randomized trials, Misirlioglu *et al*. [19], testing pentoxifylline and vitamin E in patients with non-small cell lung cancer, and Lissoni *et al*. [20], testing melatonin in patients with brain glioblastomas, found that radiotherapy combined with vitamin E or melatonin supplementation increased survival. However, Berk et al. [21] did not confirm this suggestion of radiosensitization of tumors in a randomized trial of radiation therapy and high-dose melatonin in brain metastases. During radiotherapy, hemoglobin concentration, white blood cells, and platelets counts decrease to levels lower than that of normal controls as shown in table 1. These observations seem to be a logical result of the effects of radiation on highly proliferating cells, and are compatible with those observed by others [22]. Endogenous antioxidants decrease with age, as does DNA repair capacity; cellular and blood levels of antioxidants further decrease during exposure to ionizing radiation. Human plasma proteins that contain sulfhydryl moieties, as well as dietary antioxidants, such as vitamins C and E, are radioprotective [15]. In the present study, the use of vitamins (C and E) during the course of radiotherapy did not protect the hematopoietic system against the effects of radiation; this may be attributed to inefficient dose and duration of using such combination to attenuate these changes and counteract the effects of radiation. Melatonin is a pineal hormone with multiple functions in humans, and has been shown to have radioprotective properties [23].

Melatonin has been shown to directly scavenge hydroxyl radicals, peroxy radicals, peroxy nitrite radicals and does so more efficiently than other known antioxidants [24].

However, the antioxidant effects of melatonin occur only at very high concentration; accordingly, larger doses than that recommended in the present study may produce better effects in this respect. Aspirin (acetylsalicylic acid) one of NSAIDs acts mainly by irreversible inhibition of cyclooxygenase enzyme and decrease prostaglandins synthesis. Various NSAIDs have additional possible mechanisms of action, including inhibition of chemotaxis, down-regulation of interleukin-1 production, decreased production of free radicals and superoxide, and interference with calcium-mediated intracellular events [25]. Aspirin has been known to reduce the risk of heart disease and stroke due to its effect on blood clotting [26]. It has also been shown to play a role in prevention of atherosclerosis by protecting endothelial cells from damage produced by oxygen radicals [27]. The net result of above effects of aspirin is the improvement observed in the studied hematological parameters especially Hb levels. Xanthine oxidase enzyme is known to be an important source of free radicals generating during different pathological conditions, and this may lead to lipid peroxidation, an effect which can be blocked by allopurinol [28,29].

Since we know that ionizing radiation can act as a potent stimulator of xanthine oxidase and consequently lead to increase superoxide anion production [30], so blockade of this pathway with allopurinol may be beneficial in ameliorating the damaging effects of these radicals on biomolecules.

CONCLUSION

Different types of antioxidant drugs can be used as an adjuvant treatment during radiation therapy; they may protect the hematopoietic system against the damaging effects of radiation. Aspirin and allopurinol have some protective effects, but the effect of melatonin as a radio-protective agent in this regard was superior to the others.

CONFLICTS OF INTEREST

The author(s) declare that they have no competing interests.

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