The Effect of Radiotherapy on Oxidative Stress, Biochemical and Hematological Parameters in Women with Breast Cancer

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Abstract:

This study was designed to evaluate the effect of radiation therapy on the oxidative stress, biochemical and hematological parameters in women with breast cancer. 95 women were studied, 20 healthy control women and 75 had breast cancer.

Malondialdehyde and glutathione (in erythrocytes and plasma), biochemical parameters (total plasma proteins, plasma albumin, uric acid and plasma calcium) levels, and hematological parameters (hemoglobin, white blood cell counts, platelets counts) all parameters were measured pre- and post radiation therapy for 4 weeks.

The result of this study indicates that the incorporation of radiation therapy as a way to destroy malignant cells, in addition to their therapeutic benefits, it may lead to further increase in the oxidative stress burden of cancer, manifested by increase in MDA production and glutathione depletion. Which may affect directly or indirectly some biochemical and hematological parameters as indicated by lowering plasma protein especially albumin, increases in plasma calcium level, and decreases in hemoglobin, white blood cells, and platelets levels.

These changes should be considered during radiation therapy and before administration of anticancer drugs or any other drugs because it may affect both the beneficial as well as the toxicity of these drugs.

Key words: radiation therapy, breast cancer, oxidative stress parameters, total plasma proteins, plasma albumin, uric acid, plasma calcium, hemoglobin, white blood cell counts, and platelets counts.

تأثير   على مقياس الاجهاد التاكسدي وبعض معاير الدم في ا

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أجريت هذه الدراسة لمعرفة تأثير سرطان الثدي والمعالجة الاشعاعية على الاجهاد التأكسدي وبعض معايير الكيمياء الحيوية والدموية في المرضى المصابين بسرطان الثدي قبل وبعد المعالجة الاشعاعية. أجريت هذه 95 (20   75   ). بينت الدراسة زيادة في الاجهاد التأكسدي في مرضى سرطان الثدي (زيادة المالون داي الدهايت ونقصان في الكلوتاثايون) في بلازما الدم وكريات الدم الحمراء. يزيد من حالة الاجهاد التأكسد .

أظهرت النتائج نقصان في مستوى بروتينات الدم وخاصة الالبومين وهذا النقصان يزداد مع العلاج الاشعاعي. ولوحظ أيضا زيادة في مستوى الكالسيوم وحامض البول في دم المرضى و العلاج الاشعاعي يزيد من ارتفاع مستوى الكالسيوم في الدم. ايضا انخفاض في مستوى الهيموكلوبين وكريات الدم البيضاء في المرضى وان العلاج الاشعاعي يزيد من هذا النقصان اضافة الى حدوث نقص في عدد الصفائح الدموية. هذه التغيرات التي تحدث في مرضى

سرطان الثدي وخاصة بعد العلاج الاشعاعي يجب ان تؤخذ بعين الاعتبار وخاصة عند أع   ية المضادة
للسرطان أو أي أدوية أخرى لان هذه التغيرات قد تؤثر على فعالية وسمية هذه الادوية بنفس الوقت.

Introduction:

The use of radiotherapy 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.

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 highly reactive and damaging chemical entities, leading to various types of harmful effects to the biomolecules [4].

The objectives of this study include:
* To study the effect of breast cancer on oxidative stress, some biochemical and hematological parameters.
* To study the effect of radiation therapy on the oxidative stress, some biochemical and hematological parameters.

Materials and Methods:
Patients' selection:

This study was carried out on (95) female with age ranged between (35 – 55 years), (20) healthy females served as a control group and (75) females with breast cancer after mastectomy treated firstly with systemic chemotherapy followed by local radiotherapy to the breast and chest wall. Radiation dose 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.

Sample collection and preparation:

10 ml of venous blood were collected from all subjects, before starting radiotherapy (base line value) and at the end of treatment .The collected blood samples were placed into heparinized plain tubes and centrifuged to separate erythrocytes. Erythrocytes were separated from plasma by centrifugation at 3000 rpm for 10 minute at 4 C°, the separated plasma layer was kept frozen until the time of analysis. The puffy coat over the erythrocytes was removed, and the erythrocytes were washed twice with ice cooled saline containing 2.0 mM sodium azide to inhibit catalase activity [5].

1- Measurement of erythrocyte malondialdehyde (MDA) levels:
   MDA is a byproduct of lipid peroxi-dation and it s measurement is based on the reaction of thiobarbituric acid with MDA forming TBA2- MDA adducts, according to the standard method of Stoks and Dormandy [5], which is modified by Gilbert et al [6].
   MDA concentration was calculated using a molar absorbtivity coefficient of 1.56×10^5 M cm and the results were expressed as n mol MDA/gm Hb.

2- Measurements of plasma malon-dialdehyde (MDA) levels:
   1.75 ml of saline azide was added to 0.25 ml of plasma, and the procedure is the same as that described for erythrocyte MDA, but the results were expressed as µ mole MDA/ L.

3- Measurements of erythrocytes and plasma glutathione (GSH) levels:
   Glutathione (sulfhydryl groups) contents of erythrocytes were measured according to the method of Godin et al[7].

4- Measurement of hemoglobin (Hb) levels:
   Hemoglobin (Hb) levels were estimated according to the method of Drapkin and Austin [8].

5- Total plasma protein level was determined according to (Kingsley [9]. and Henry [10] Results were expressed in gm/ dl.

6- Determination of plasma albumin levels according to (Doumas *et al* [11]. Results were expressed in gm/dl.

7- Determination of plasma uric acid levels according to Watts [12]. Results were expressed in mg/dl.

8- Plasma calcium levels were determined according to the method of Lorentz[13]. Results were expressed m mol/ L.

9- WBC and platelets counts were determined by using automated analyzer MS9 instruments (MELETS & Schloesing HLOESING laboratories).

10- Statistical analysis:

  A- The results were expressed as mean ± S.E.

  B- Student T-test and ANOVA test were used to examine the degree of significance and P value less than 0.05 was considered significant.

Results:

1-Effect of breast cancer and radiotherapy on the oxidative stress parameters:

As shown in table (1), breast cancer produces significant elevation in erythrocytes and plasma malondialdehyde levels compared with normal controls (87% and 41% respectively). Further elevation in malondialdehyde levels in both compartments were observed in table (1) as a result of exposure to radiotherapy (82% in erythrocytes and 82% in plasma) and found to be significantly different compared to pre-radiation.

Table (1) also demonstrated that erythrocytes and plasma glutathione levels were significantly decreased in breast cancer patients compared to healthy controls (7% and 13% respectively). Further reduction in glutathione levels in both compartments was observed in table (1) as a result of exposure to radiation (33% in erythrocytes and 31% in plasma), and these values were found to be significantly different compared to pre-radiation values.

2-Effect of breast cancer and radiotherapy on plasma proteins:

As indicated in table (2), breast cancer produces significant reduction in plasma total protein levels (p<0.05) compared to controls (9%), and radiotherapy did not significantly affect these values. Meanwhile, table (2) demonstrated also a significant reduction in plasma albumin levels (15%) compared to controls, and further reduction (10%) was observed after radiotherapy, and found to be significantly different with respect to pre-radiation stage.

3- Effect of breast cancer and radiotherapy on plasma uric acid and calcium level:

Table (3) demonstrated that both uric acid and calcium were significantly elevated as a result of breast cancer (p<0.05) (20% and 26% respectively) compared with normal controls, radiotherapy show no significant changes in uric acid levels, while plasma calcium levels were significantly elevated (18%) compared to pre-radiation values.

4- Effect of breast cancer and radiotherapy on some hematological parameters:

As shown in table (4), where hemoglobin levels and white blood cells count were found to be significantly reduced (3% and 19% respectively) compared to normal controls, while no changes were observed in platelets count.

As a result of radiotherapy all the studied parameters in this respect were significantly reduced (p<0.05) compared with pre-treatment with radiotherapy levels (6% for hemoglobin, 21% for white blood cells count and 16% for platelets count).

Table-1: **Effect of radiotherapy on the oxidative stress parameters (MDA and GSH) in Erythrocytes and plasma of breast cancer patients.**

parameters	Control n = 20	Cancer patients Pre-treatment with radiotherapy n = 75	Radiotherapy treated patients	
			Before Radiotherapy n = 15	After Radiotherapy n = 15
Erythrocytes MDA (n mole/ g Hb)	15.53 Ë 0.55	29.08 Ë 1.30†	19.22 Ë 2.19	35.05 Ë 4.23** †
Plasma MDA (~ mole/ L)	1.69 Ë 0.07	2.38 Ë 0.13†	1.48 Ë 0.24	2.69 Ë 0.24** †
Erythrocytes GSH (~ mole/ g Hb)	8.06 Ë 0.16	7.47 Ë 0.24†	8.57 Ë 0.48	5.77 Ë 0.43** †
Plasma GSH (~ mole/ L)	0.23 Ë 0.01	0.20 Ë 0.01†	0.16 Ë 0.02	0.11 Ë 0.01** †

Each value represents mean Ë S.E.
n = number of subjects
† Significantly different with respect to control ($p < 0.05$)
**** Significantly different with respect to treated patients before radiotherapy ($p < 0.05$)**

Table -2: Effect of radiotherapy on the total plasma protein and albumin levels in breast cancer patients.

parameters	Control n = 20	Cancer patients Pre-treatment with radiotherapy n = 75	Radiotherapy treated patients	
			Before Radiotherapy n = 15	After Radiotherapy n = 15
Total plasma protein (gm/ dl)	7.73 Ë 0.07	7.06 Ë 0.28†	7.52 Ë 0.35	7.12 Ë 0.50†
Plasma albumin (gm/dl)	4.22 Ë 0.07	3.58 Ë 0.11†	4.04 Ë 0.20	3.64 Ë 0.18** †

Each value represents mean Ë S.E.
n = number of subjects
† Significantly different with respect to control ($p < 0.05$)
**** Significantly different with respect to treated patients before radiotherapy ($p < 0.05$)**

Table-3: Effect of radiotherapy on plasma levels of uric acid and calcium in breast cancer patients.

Parameters	Control n = 20	Cancer patients Pre-treatment with radiotherapy n = 75	Radiotherapy treated patients	
			Before Radiotherapy n = 15	After Radiother-apy n = 15
Plasma uric acid (mg/ dl)	3.74 ± 0.11	4.50 ± 0.18†	4.34 ± 0.47	4.47 ± 0.49
Plasma calcium (m mole/ L)	2.58 ± 0.08	3.26 ± 0.09†	2.90 ± 0.15	3.41 ± 0.19** †

Each value represents mean ± S.E.

n = number of subjects

† Significantly different with respect to control (p < 0.05)

** Significantly different with respect to treated patients before radiotherapy (p < 0.05)

Table-4: Effect of radiotherapy on the hematological parameters in breast cancer patients.

parameters	Control n = 20	Cancer patients Pre-treatment with radiotherapy n = 75	Radiotherapy treated patients	
			Before Radiotherapy n = 15	After Radiotherapy n = 15
Hemoglobin (gm/ dl)	11.48 ± 0.09	11.13 ± 0.12†	11.28 ± 0.26	10.59 ± 0.22** †
WBC count (10^9/L)	5.96 ± 0.18	4.83 ± 0.12†	5.03 ± 0.29	4.00 ± 0.26** †
Platelet count (10^9/L)	195.53 ± 2.95	190.51 ± 3.01	201.25 ± 10.00	170.00 ± 17.18** †

Each value represents mean ± S.E.

n = number of subjects

† Significantly different with respect to control (p < 0.05)

** Significantly different with respect to treated patients before radiotherapy (p < 0.05)

Discussion:

The data presented in this study (table-1) very well showed that a state of systemic oxidative stress, manifested by an elevation in MDA levels and depletion of glutathione in erythrocytes and plasma is a common feature in all breast cancer patients included in this study. It was found that systemic oxidative stress is thought to contribute as a risk factor for various cancers, including breast cancer[14].

The results of this study, table-1 showed that total MDA levels in both erythrocytes and plasma of breast cancer patients were significantly higher than those of controls; these data are compatible with those observed by others [15]. The elevated MDA levels reflects an increase in the process of lipid peroxidation, where the pathogenicity of the disease may be resulted from or ending with oxidative stress due to exaggerated production of reactive oxygen species and other

metabolites leading to a cellular damage at the molecular levels which may end up with the cell proliferation and malignant conversion [16].

The total antioxidant status is found to be reduced in patients with various types of tumors compared with healthy controls[17] and many epidemiological studies have shown that low antioxidants levels were associated with the increased incidence of certain cancers.

The results of this study clearly showed that the antioxidant status, expressed as a glutathione levels in erythrocytes and plasma, was severely impaired in breast cancer patients compared to controls, and radiotherapy worsen the case, (table-1), and low glutathione levels could be explained both by the increased neutralization of free radicals as a result of cancer pathogenesis and the decrease in the turnover synthesis and recycling of this important endogenous antioxidant. These finding are compatible with those observed by Sardesia [18].

Total plasma protein and especially albumin, due to its high content of thiol groups, can function as antioxidant system through a chain breaking effect to terminate the chain reaction of lipid peroxidation. This neutralization of the free radicals can result in the formation of thinly radicals [19].

The present study demonstrated clearly the relationship between the elevated levels of lipid peroxidation and lowering of both plasma total protein and albumin, where significant reduction was observed in both cases, cancer patients' pre- and post- radiotherapy compared to controls (table-2).

During exposure to radiation, oxygen radicals produced a dramatic change in the structure and functions of plasma proteins, and it is found that free radicals- induced conformational changes of bovine serum albumin was associated with the impairment of its antioxidant properties [20], and may result in dramatic changes in protein stability and functions

but these oxidative processes can be inhibited by the use of high doses of the chain–breaking antioxidants [21].

Another pathway for the loss of plasma protein is through the increase in capillary permeability due to the reactive oxygen species induced changes in the endothelium of the blood vessels; these will lead to the escape of albumin to the interstitial compartment [22].

The single element that has received the most attention as a mediator of cell injury is calcium [23]. Any perturbation that may affect calcium transport across plasma membrane is capable of seriously affecting cell functions, especially when we know that the calcium-ATPase has a critical thiol groups and can be inactivated by reactive oxygen species [24]. In addition to that reactive oxygen species mediated damage can affect the energy required to maintain calcium homeostasis (and other ions), leading to disturbances in the gradients required for the normal levels [25].

The results of this study, table-3 show a highly significant elevation in plasma calcium levels in breast cancer patients before and after radiotherapy, this could be explained on the basis of increased reactive oxygen species generation during both cancer pathogenesis and radiotherapy. Other studies showed that radiation can result in an increased penetration of extra-cellular calcium into the cytoplasmic compartment [26].

It was found that 40% of plasma calcium is bound to plasma protein especially with albumin [27,28] the reduction of plasma albumin concentration observed in this study, table (2), in both pre- and post- radiotherapy of breast cancer patients is found to affect plasma calcium levels[29].

Uric acid is the end-product of purine metabolism, it is an efficient free radical scavenger, being converted as a result of scavenging process to allantoin[30], part of the antioxidant activity of urate might be attributed to the

formation of a stable, non–reactive complex with iron, but it has a direct free radical scavenging activity [31,32].

In table-3, Uric acid is found to be significantly elevated in cancer patients compared to normal controls, and this can be attributed to the increased nucleic acid turnover and consequent increase in the catabolism of purine bases as result of rapid proliferation of tumor cells, as well as massive destruction of surrounding tissues [33].

The non significant change in the urate levels as a result of exposure to radiotherapy may be related with the increase in the urinary excretion (not measured) or excessive utilization of these antioxidant molecules in scavenging of the excessively produced reactive oxygen species during radiation [34].

During radiotherapy, hemoglobin and white blood cells level may decrease to levels lower than that of normal controls, (table-4).

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[35,36]. The same pattern of changes was observed for the platelets as a part for the hematopoietic system.

In conclusion, radiation therapy initiates, ionization leading to free radical formation which affects antioxidant defense mechanisms of the body, aggravating the already present state of oxidative stress induced by cancer pathophysiology. These biochemical and hematological changes induced by cancer and radiation therapy as shown in this study should be considered before administration of anticancer drugs or any other drugs, because it may affects the beneficial as well as the toxicity of these drugs.

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