REPORTS OF PRACTICAL ONCOLOGY AND RADIOTHERAPY 21 (2016) 141–148

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/rpor

Original research article

Pre and post radiotherapy serum oxidant/antioxidant status in breast cancer patients: Impact of age, BMI and clinical stage of the disease

Maryam Khalil Arjmandi[a], *Dariush Moslemi*[b], *Azadeh Sadati Zarrini*[c], *Maryam Ebrahimnezhad Gorji*[c], *Abbas Mosapour*[a], *Asefeh Haghhaghighi*[a], *Sohrab Halalkhor*[a], *Ali Bijani*[d], *Hadi Parsian*[e,*]

[a] *Department of Clinical Biochemistry, Babol University of Medical Sciences, Babol, Iran*
[b] *Department of Radiation Oncology, Babol University of Medical Sciences, Babol, Iran*
[c] *Department of Biology, Science and Research Branch, Islamic Azad University, Fars, Iran*
[d] *Social Determinant of Health Research Center, Babol University of Medical Sciences, Babol, Iran*
[e] *Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*

ARTICLE INFO

Article history:
Received 30 May 2015
Received in revised form
4 November 2015
Accepted 23 December 2015

Keywords:
Breast cancer
Oxidative stress
Radiotherapy
Selenium
Total antioxidant status

ABSTRACT

Aim: In this study the effects of radiation therapy (RT) on serum oxidant/antioxidant status in breast cancer patients and the impact of age, BMI and clinical stage of the disease on the aforementioned variables were investigated.
Background: RT that is used for cancer treatment is dependent on the production of reactive oxygen species.
Materials and methods: Eighty patients with breast cancer participated in this study and received RT at a dose of 50 Gy for 5 weeks. Blood samples were obtained in one day before and after the end of RT. Serum status of malondialdehyde (MDA), total antioxidant status (TAS), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were analyzed by spectrophotometry or ELISA and selenium (Se) level were analyzed by atomic absorption spectrometry. Paired *t*-test was used for comparing pre and post radiotherapy data.
Results: Before and after the radiotherapy, a significant increase in MDA level was observed, while a significant decrease in GPx activity, SOD, TAS and Se levels were found ($p < 0.05$). The level of the CAT enzyme had no significant changes ($p = 0.568$). The results showed some changes in the status of TAS, SOD and GPx which are associated with age, BMI and clinical stage of the disease.

* *Corresponding author at*: Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Ganjafrooz Ave, Babol, Iran. Tel.: +98 1132199936; fax: +98 1132199936.
E-mail address: hadiparsian@yahoo.com (H. Parsian).
http://dx.doi.org/10.1016/j.rpor.2015.12.009

Conclusion: It seems that RT would have the potential to cause variations in the status of antioxidant/oxidant system. Although, some changes in variables were observed by sub-classification of the age, BMI and the disease stage, but it seems that these changes are not necessarily dependent to them.

1. Background

Cancer is the second cause of mortality worldwide and breast cancer is the most prevalent cancer in women, comprising 29 percent of all cancers that they are affected with.[1] It is also worth mentioning that more than one million women are diagnosed with breast cancer annually.[2] According to statistics, the incidence rate of this disease is comparatively low in Asian and African countries, but it is increasing more rapidly in comparison with other countries.[3] Although breast cancer is more prevalent in people older than 50 years, in Iran, the incidence rate of the disease seems to be lower than in the other countries.[4]

The exact etiology of breast cancer has not been completely understood, so it is assumed as a multifactorial disease. Several studies suggested that exposure to various carcinogens would increase the risk of cancer and the best known among the possible causes of breast cancer development are inflammatory factors, nutritional parameters, obesity and genetic mutations.[5,6]

Currently, surgery, radiotherapy (RT), chemotherapy and hormone therapy are effectively used for the treatment of cancer patients.[7,8] RT, as one of commonly used methods for the treatment of cancer, is able to destroy remaining cancer cells after surgery.[9] Patients with cancer need RT either for curative or palliative purposes, but unfortunately, this valuable method is associated with some major side effects.[10] It has been shown that the risk of heart disease would increase after radiation in these patients.[11,12] Although, in RT, the aim is to affect just the target organs, skin, bone marrow hematopoietic cells and the other organs with rapid proliferation rate will also be damaged.[13–16]

Radiation therapy leans on ROS (reactive oxygen species) toxicity and can damage cellular macromolecules, such as DNA (deoxyribonucleic acid), RNA (ribonucleic acid), microRNAs, proteins and membrane in tumor cells.[17–19] Antioxidants protect normal cells against radiation injury through various enzymatic systems, such as catalase, glutathione peroxidase and superoxide dismutase. In addition, normal cells would benefit from non-enzymatic systems (such as selenium, glutathione and tocopherol) to scavenge the free radicals.[20,21] It has been suggested that radiation can cause a decline in the level of vitamins A, E, C and selenium in breast cancer patients.[22,23]

2. Aim

There are some data regarding the effects of RT on various antioxidant/oxidant systems, but the results do not seem to be conclusive. Some studies have reported that RT is able to change the status of the antioxidant/oxidant system,[23–27] while other reports have pointed out this treatment does not have big effects on the antioxidant/oxidant system.[23,28,29] Therefore, in this study, we aimed to determine the exact effects of RT on the oxidant/antioxidant status, the activity of glutathione peroxidase (GPx) and the levels of catalase (CAT), superoxide dismutase (SOD) and selenium (Se) in a group of women with breast cancer. To this end, we followed all the patients before and after RT to determine the effects of this type of treatment on aforementioned variables. In addition, we determined the impact of age, BMI and clinical stage of the disease on the above mentioned biochemical variables.

3. Materials and methods

3.1. *Study population*

This study was performed on 80 women with breast cancer approved by pathological examinations. The demographic and clinical data of the patients, including age, clinical stage, body mass index (BMI) and histological grade were recorded for each patient. The stage of breast cancer was determined by the tumor node metastasis (TNM) system.[30] Forty-five patients with early stage of breast cancer (stage I and II) and 35 patients

Table 1 – Characteristics of breast cancer patient included in this study.

Mean ± SD		*n* (%)
Age (years)	50.42 ± 11.1	80 (100)
BMI (kg/m^2)	30.22 ± 5.26	80 (100)
Clinical stage		
Stage I		8 (10)
Stage II		37 (46.3)
Stage III		31 (38.8)
Stage IV		4 (5)
Estradiol receptor		
Positive		58 (72.5)
Negative		22 (27.5)
Progesterone receptor		
Positive		58 (72.5)
Negative		22 (27.5)
Her-2		
Positive		32 (40)
Negative		48 (60)
Histology type		
IDC		75 (93.8)
ILC		5 (6.2)

Notes: Age and BMI are expressed as mean ± standard deviation (SD); BMI: Body mass index (kg/m^2); Her-2: human epidermal growth factor receptor; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma.

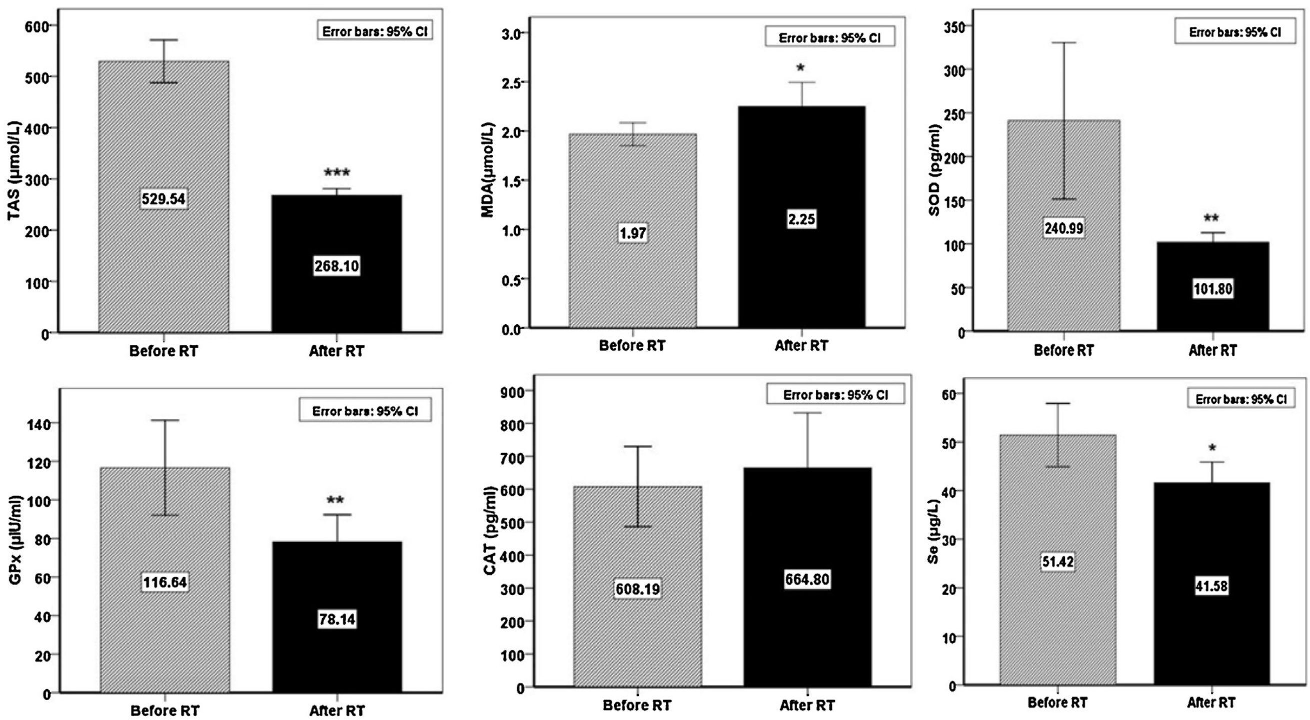

Fig. 1 – Serum levels of oxidant/antioxidant status before and after radiotherapy in breast cancer. Note: TAS: total Antioxidant status; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; Se: selenium. Data expressed as mean ± SD; $p < 0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Table 2 – Serum levels of oxidant/antioxidant status before and after radiotherapy in different age groups.

Variables		Age ≤50 years old ($n = 43$)	Age >50 years old ($n = 37$)	p value
TAS (μmol/L)	Before RT	505.8 ± 187.1	559.3 ± 190.9	0.938
	After RT	262.8 ± 55.2	274.6 ± 62.5	0.332
	p value	<0.001	<0.001	
MDA (μmol/L)	Before RT	2.0 ± 0.5	1.9 ± 0.5	0.385
	After RT	2.3 ± 1.1	2.3 ± 1.0	0.709
	p value	0.141	0.111	
SOD (pg/ml)	Before RT	184.2 ± 250.7	315.4 ± 250.7	0.012
	After RT	92.1 ± 39.9	112.4 ± 59.4	0.005
	p value	0.012	0.022	
CAT (pg/ml)	Before RT	492.6 ± 461.6	721.1 ± 628.5	0.015
	After RT	598.8 ± 459.2.0	726.0 ± 1002.4	0.370
	p value	0.308	0.979	
GPx (μIU/ml)	Before RT	103.3 ± 85.2	135.5 ± 137.6	0.080
	After RT	68.4 ± 42.1	90.2 ± 83.3	0.138
	p value	0.008	0.034	
Se (μg/L)	Before RT	53.5 ± 32.8	49.7 ± 26.6	0.418
	After RT	40.3 ± 17.0	42.6 ± 22.5	0.767
	p value	0.027	0.259	

Notes: TAS: total Antioxidant status; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; Se: selenium; RT: radiotherapy; data expressed as mean ± SD; $p < 0.05$.

with advanced breast carcinoma (stage III and IV) were participated in this study. All patients were submitted to RT three weeks after the chemotherapy period; while, in the beginning of the study, they had normal values of hematological/biochemical parameters. They received RT at a dose of 50 Gy with fraction size of 2 Gy for 5 weeks (five days weekly). The blood samples were obtained from all patients one day before and after the end of RT treatment. The samples were centrifuged and the resulted serums were stored at −80 °C until the final analysis.

Table 3 – Serum levels of oxidant/antioxidant status before and after radiotherapy in different BMI groups.

Variables		BMI ≤30 kg/m² ($n=42$)	BMI >30 kg/m² ($n=38$)	p value
TAS (μmol/L)	Before RT	546.0 ± 190.6	512.7 ± 189.2	0.472
	After RT	261.9 ± 62.4	275.3 ± 54.0	0.500
	p value	<0.001	<0.001	
MDA (μmol/L)	Before RT	2.0 ± 0.5	1.9 ± 0.5	0.999
	After RT	2.3 ± 1.1	2.2 ± 1.1	0.881
	p value	0.152	0.106	
SOD (pg/ml)	Before RT	201.3 ± 288.0	293.1 ± 516.2	0.165
	After RT	99.5 ± 45.7	103.8 ± 56.0	0.165
	p value	0.017	0.024	
CAT (pg/ml)	Before RT	651.5 ± 548.7	543.6 ± 562.6	0.713
	After RT	657.5 ± 941.1	657.8 ± 494.2	0.892
	p value	0.950	0.406	
GPx (μIU/ml)	Before RT	106.1 ± 103.9	131.5 ± 22.2	0.456
	After RT	73.6 ± 43.2	83.8 ± 83.0	0.135
	p value	0.046	0.009	
Se (μg/L)	Before RT	51.4 ± 25.5	52.2 ± 34.5	0.284
	After RT	43.5 ± 21.8	39.0 ± 16.8	0.557
	p value	0.144	0.053	

Notes: BMI: Body mass index (kg/m²); TAS: total antioxidant status; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; Se: selenium; RT: radiotherapy; data expressed as mean ± SD; $p < 0.05$.

3.2. *Measurement of oxidant/antioxidant status*

In this study, several biochemical parameters of the oxidant and antioxidant status were evaluated. Serum total antioxidant status (TAS) was analyzed according to the FRAP (ferric reducing ability of plasma) assay. In this test, regeneration of ferric to ferrous ions in the presence of antioxidants in low pH, produces a colored complex: Fe (II)-TPTZ (Ferrous-Tri-pyridyl-Striazine) with a maximum absorbance at 593 nm. Absorbance changes in the serum samples and standards were measured and expressed in μmol/L.[31]

Lipid peroxidation, as the level of malondialdehyde (MDA), was analyzed with the thiobarbituric acid reactive substances (TBARS) assay. The principle of this assay is to determine lipid hydroperoxids levels by spectrophotometer. After the reaction of MDA with two molecules of thiobarbituric acid and

Table 4 – Serum levels of oxidant/antioxidant status before and after radiotherapy in different clinical stage of disease.

Variables		Early stage ($n=45$)	Advanced stage ($n=35$)	p value
TAS (μmol/L)	Before RT	540.2 ± 184.8	516.3 ± 197.3	0.694
	After RT	270.2 ± 58.4	268.9 ± 58.1	0.486
	p value	<0.001	<0.001	
MDA (μmol/L)	Before RT	2.0 ± 0.5	1.9 ± 0.5	0.443
	After RT	2.4 ± 1.2	2.1 ± 1.0	0.424
	p value	0.032	0.499	
SOD (pg/ml)	Before RT	215.4 ± 367.7	282.7 ± 466.2	0.243
	After RT	100.6 ± 52.4	102.6 ± 48.8	0.905
	p value	0.034	0.020	
CAT (pg/ml)	Before RT	541.9 ± 480.3	672.2 ± 635.5	0.031
	After RT	503.1 ± 314.0	856.3 ± 1064.9	0.021
	p value	0.671	0.357	
GPx (μIU/ml)	Before RT	114.3 ± 103.2	123.1 ± 125.7	0.619
	After RT	83.1 ± 78.1	72.5 ± 43.0	0.244
	p value	0.036	0.012	
Se (μg/L)	Before RT	50.4 ± 28.5	53.5 ± 32.1	0.271
	After RT	40.6 ± 16.9	42.3 ± 22.8	0.642
	p value	0.053	0.130	

Notes: TAS: total antioxidant status; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; Se: selenium; RT: radiotherapy; data expressed as mean ± SD; $p < 0.05$.

the elimination of two water molecules, the amount of pink-colored complex was measured at 532 nm and the results were expressed in μmol/L.[32]

3.3. Assay of antioxidant enzymes

The levels of SOD and CAT as well as GPx activity were measured by ELISA kit on an ELISA reader (Awareness – America). All enzymes were analyzed by CUSABIO ELISA kit, China (catalogue numbers: CSB-EL022399HU, CSB-E13635 and CSB-E09496h, respectively for SOD, CAT and GPx) employing the quantitative sandwich enzyme immunoassay technique. All analyses were performed according to the manufacturer's instruction. The CAT and SOD levels were expressed in (pg/ml) and GPx activity was expressed in (μIU/ml).

3.4. Selenium measurement by atomic absorption spectrometry (AAS)

The serum selenium concentrations were determined by atomic absorption spectrometry (PG-990, china) with the graphite analysis technique. Analytical parameters used for this procedure were: λ: 196 nm, bandwidth: 2 nm and element lamp current: 5 mA. A standard solution was made up from stock solution of Seo2 (Merck, 1000 ppm) and the standard curves were plotted by selenium concentrations of 0, 12.5, 25, 50, 100 and 200 μg/l (ppb). The samples were diluted in a ratio of 1/2 with 0.1% nitric acid (Merck) in order to avoid precipitation, then 10 μl of the diluted samples was injected into the graphite furnace and the results were expressed in ppb.

3.5. Statistical analysis

Statistical analyses were performed using the SPSS version 17 (PASW, USA). Demographic and clinical data were expressed as mean ± SD. The paired *t*-test was used for comparing pre and post RT data and statistical significance was considered as $p < 0.05$.

4. Results

Patient's demographic and clinical data has been summarized in Table 1. Fig. 1 shows serum levels of the antioxidant/oxidant status, antioxidant enzymes and Se, before and after the RT. The results showed a statistically significant decrease in TAS, SOD levels and GPx activity as well as serum Se concentration ($p < 0.05$). In contrast, the MDA level ($p = 0.047$) showed a significant increase in these patients and there were no significant difference in CAT enzyme level ($p = 0.568$) before and after the RT.

Next, all of the analyzed variables were categorized in different age groups (≤ 50 and >50 years old), BMI (≤30 and >30 kg/m^2) and the clinical stages of the disease were considered as early and advanced stages.

Between the two age groups, there were no significant differences in MDA and CAT levels before and after the RT (Table 2). In patients with age ≤50 years, RT led to a significant decrease in GPx activity and SOD level. In addition, in these patients, TAS and Se concentrations decreased significantly.

Table 5 – Serum levels of oxidant/antioxidant status before and after radiotherapy according to the progesterone receptor, estradiol receptor and Her-2 receptor.

Variables		PR positive (n = 58)	PR negative (n = 22)	p value	ER positive (n = 58)	ER negative (n = 22)	p value	Her-2 positive (n = 32)	Her-2 negative (n = 48)	p value
TAS (μmol/L)	Before RT	517.3 ± 189.5	555.8 ± 192.3	0.444	513.0 ± 184.0	568.7 ± 205.2	0.543	527.1 ± 199.8	527.0 ± 184.8	0.994
	After RT	267.3 ± 59.7	284.3 ± 61.7	0.543	267.7 ± 59.9	283.1 ± 61.6	0.771	258.3 ± 53.0	280.7 ± 63.8	0.669
	p value	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	
SOD (pg/ml)	Before RT	236.2 ± 432.5	262.7 ± 350.9	0.515	243.0 ± 433.1	244.3 ± 350.9	0.864	270.9 ± 477.8	225.3 ± 363.1	0.991
	After RT	96.8 ± 48.5	111.5 ± 55.7	0.751	96.7 ± 49.6	106.4 ± 54.1	0.536	104.9 ± 51.8	98.1 ± 50.2	0.283
	p value	0.012	0.037		0.01	0.055		0.048	0.012	
GPx (μIU/ml)	Before RT	120.0 ± 115.0	115.3 ± 107.3	0.911	120.5 ± 114.8	113.1 ± 108.0	0.903	117.1 ± 115.0	19.8 ± 111.8	0.854
	After RT	68.4 ± 42.1	68.4 ± 54.4	0.934	83.5 ± 68.9	65.4 ± 50.1	0.828	83.0 ± 49.1	75.6 ± 73.3	0.842
	p value	0.009	0.031		0.01	0.023		0.1	0.02	

Notes: PR: progesterone receptor, ER: estradiol receptor, Her-2: human epidermal growth factor receptor, TAS: total antioxidant status; SOD: superoxide dismutase; GPx: glutathione peroxidase; RT: radiotherapy; data expressed as mean ± SD; $p < 0.05$.

In older patients, the RT significantly decreased the serum level of SOD, TAS and also GPx activity, whereas this type of treatment had not a significant effect on Se, MDA and CAT levels. Generally, in older age groups before and after the RT, higher levels of SOD, CAT, TAS and GPx activity were observed. It seems that the level of SOD is age dependent, as older age groups indicate higher levels ($p = 0.012$) maintaining this trend also after RT ($p = 0.005$), but such a trend was observed in serum CAT level only before the RT ($p = 0.015$).

Comparative analyses of the determined variables in two BMI groups are shown in Table 3. There were no significant differences in MDA and CAT levels, before and after the RT in both groups, but the mean of SOD level, GPx activity and TAS level decreased significantly after the RT in two groups. We observed that the serum concentration of Se in patients with higher BMI decreased more after the RT in comparison to the patients with BMI $\leq 30\,kg/m^2$.

Stages I and II of the breast cancer were considered as early stage and stages III and IV of breast cancer as advanced stages of the breast cancer. The results indicated that the patients with advanced stages of breast cancer had higher levels of CAT levels. The effects of RT on the measured variables were compared in these two stages of disease and five weeks after the RT, CAT level showed no statistically significant difference in comparison with pretreatment levels in the two groups (Table 4). The level of TAS in both groups was decreased significantly. It seems that the RT, also caused a serum Se concentration decrease (but not significant) according to the crude data of both stages. An increase in the level of MDA was observed in both stages of the disease after RT, but only in the early stage differences were significant. The results of this study also revealed a significant decrease in SOD level and GPx activity after the RT in both stages of the disease. Table 5 shows serum levels of the oxidant/antioxidant status before and after radiotherapy according to the progesterone receptor, estradiol receptor and Her-2 receptor.

5. Conclusions

Significant differences were observed in the status of MDA, TAS, SOD, GPx and Se before and after RT. The levels of MDA were increased, while the other variables were decreased. No significant difference was observed between pre- and post-radiotherapy CAT enzyme levels in the patients. RT led to a significant decrease in GPx activity, SOD and TAS levels, meanwhile a non-significant increase was observed in the MDA and CAT levels in the two groups of age (≤ 50 and >50). In younger patients, Se concentration decreased after the RT ($p = 0.027$), whereas there was no significant difference in the serum Se level of older ones. A similar pattern was observed for the status of analyzed biochemical variables in the two BMI groups (≤ 30 and >30). Se levels showed a decrease in crude data after the RT in these two groups, but the difference was not significant. A significant decrease in GPx, SOD and TAS status were observed in both two stage groups of patients (early and advanced stages) and the others had no significant differences after the treatment.

As previously mentioned, radiation therapy relies on ROS production and toxicity. Antioxidants protect normal cells against radiation injury. As in the patients with cancer, all aspects of normal metabolism would be disturbed; therefore, these patients are susceptible to dyshomeostasis in various metabolic fields. When the patients are treated with radiation, they would experience another dyshomeostatsis in their metabolic conditions which adversely affects the disease status. Therefore, returning the patients to the normal state is extremely recommended. In our study, it was observed that some biochemical variables were affected by RT, while the others were not.

Investigation of RT effects on the oxidant and anti-oxidant status in various cancers, such as breast cancer, is valuable. In a study by Belwalkar et al. on women with breast cancer, the level of MDA elevated; SOD activity and vitamin E were lower before and after radiation therapy than those of healthy controls ($p < 0.001$).[24] Alterations in serum Se concentration have been shown in breast cancer patients after the radiation. Franca et al. evaluated the effects of RT on plasma Se concentration in 209 breast cancer patients and reported a significant reduction in the plasma selenium concentration ($p < 0.001$) for the patients undergoing RT.[7] The role of SOD in breast cancer blood cells was also investigated by Pajovic et al.[25] The authors stated that alterations in the SOD activity is directly related to the initiate status of this enzyme in the conditions of high baseline level of SOD activity, RT would decrease this activity, but in conditions in which the primary activity is medium or low, the activity would be approximately constant or might even increase after the RT. As another example, in the study carried out by Kasapovic et al. on breast cancer patients, RT significantly increased the activities of SOD and CAT enzymes, but there were no steady changes in GPx activity in these patients.[26] In another study on 58 breast cancer patients after chemotherapy, but not RT, an increase in the level of lipid hydro peroxides and a decrease in SOD, GPx and glutathione level were reported in these patients.[27] The above findings are similar to the study performed by Thanoon et al. on MDA and SOD levels in 23 breast cancer patients. They showed that chemotherapy treatment caused a reduction in serum TAS and led to an elevation in serum MDA ($p < 0.001$).[33] Bindary et al. determined the status of SOD and CAT levels in 50 patients with breast cancer, before and after chemotherapy they reported that the levels of both variables were significantly lower than before the treatment ($p < 0.001$).[34]

There are also some controversial reports in the literature, such as Khoshbin et al., that investigated the effect of RT and chemotherapy on MDA level in breast cancer patients. They did not observe a statistically significant difference between the levels of MDA during the chemotherapy/RT period.[28] In another report by Nwozo et al. on 30 breast cancer patients with either chemotherapy or RT, a non-significant decrease in SOD activity and a significant increase in lipid peroxidation were reported in comparison with healthy controls.[29] In addition to the published article regarding RT effects on patients with breast cancer, there are some reports that analyzed these changes in other cancers. Sabitha in a study on oral cancer patients evaluated the status of SOD, CAT, GPx and MDA levels and reported that the radiation may cause a decrease in enzymes activity and an increase in lipid peroxidation products.[21] Baranwal et al. in a study on 80 patients with anaplastic astrocytoma showed significant changes in

MDA (increase) and GPx (decrease) after RT.[35] Similarly, Crohns et al. on 36 lung cancer patients showed that the level of total lipid peroxidation products increased significantly after RT.[36] Again, there are some data reported that the RT has no remarkable effects on various oxidant/antioxidant status in the other cancers. Wozniak et al. in their research on patients with prostate cancer reported no significant differences in SOD and CAT activity, as well as MDA concentration before and after radiation therapy.[37] In another survey by Demirci et al. on antioxidant status in 35 cervical cancer patients, the authors did not find significant differences between the levels of SOD, MDA and GPx status pre-RT and post-RT.[38]

According to our knowledge, the reports regarding to the impact of age on the oxidant/antioxidant variables are limited. As previously mentioned in two age groups of patients, RT led to a significant decrease in some variables (GPx, SOD and TAS) and a non-significant increase in MDA and CAT levels. In addition, in our series the younger patients experienced a significant decrease in Se concentration after RT as compared to the older ones. Interestingly, the levels of Se in older patients were lower than the younger's ones. It seems that the level of this trace element is age-dependent. Perhaps older patients need to intake more essential minerals, especially Se, which can be provided as a supplement or in the foods that contain it. The study of Franca et al. is consistent with our study as they showed a significant lowered selenium levels in breast cancer patients with advanced age (>60), compared before and after the RT.[7] As we know, Se plays very important roles in a number of biological processes. On the other hand, it is a component of Gpx enzyme and alteration in glutathione peroxidase activities depended on the serum Se concentration.[21] Lower selenium levels have been observed in patients with previous chemotherapy, low BMI (consistent with our study) and advanced age as well as advanced stage, before the treatment.[7]

The result of the present study also demonstrated a significant decrease in GPx, SOD and TAS in two BMI groups. There are several studies indicating that patients with higher BMI had lower prognosis. Some authors proposed that maybe these patients had been diagnosed in advanced stages.[39–41] In our opinion, in addition to the late diagnosis and having more aggressive tumors, lower levels of TAS and some enzymes, such as CAT, would be the reason, as it was observed in our study.

In two stage groups of the patients, RT led to a significant decrease in GPx, SOD and TAS. In addition patients in early stage experienced a significant increase in MDA after RT as opposed to those in advanced stages. It seems that in the early stage of the disease, ROS formation would suddenly increase. After the disease progresses to higher stages, the body tries to compensate the occurring irregularities and the antioxidant enzymes, such as CAT, GPx and SOD, are expressed more to overcome the overproduction of MDA in advanced stages, as was observed in our study. The result of this study is in contrast with Belwalkar et al. findings on breast cancer that reported MDA level decreased in the early stage and increased in the advanced stages.[16]

The RT that is used as a common treatment option for breast cancer patients had a lot of effects on various metabolism pathways in the body. As we observe in our study, it had effects on the oxidant/antioxidant system. Usually, it is expected that when oxidative stress increases, antioxidant should be decreased, but our study showed that these two systems would not necessarily follow that pattern. It seems that when the normal metabolism of the body is disturbed, it should not be expected for all the harmful parameters to increase and all the useful parameters to decrease. It seems that after dyshomeostasis of the normal metabolism, either caused by a disease or by treatment interventions (for example RT), a big imbalance would occur in the cell which can lead to adverse changes in the disease status. Normally, it is expected that the resistance power of young patients is different to those of the old ones and it seems that patients in the higher stages of the disease would be more involved and might also have severe inconsistency in their cells. In all these conditions, we assume that body behaves like a regular known machine, while our body is a complex and really unknown machine. Therefore, it can be expectable that when an imbalance happens in the cells, all variables show different patterns of changes and this is the reason why we are not able to prevent and/or treat various spectra of the disease, yet.

In conclusion, the results showed that in young patients with breast cancer, RT led to a decrease in the serum selenium level and, interestingly, it seems that higher BMI can intensify this condition. In addition, it seems that patients in higher stages of the disease are more susceptible to the dyshomeostasis of antioxidant capacity after RT. Therefore, it is suggested that patients with lower age and higher BMI should consider a specific attention in nutritional and antioxidant supplementation. As a result, increased level of antioxidant is probably able to decrease the rate of normal cell transformation toward malignancy and ultimately increase the patient survival rate.

Conflict of interest

None declared.

Financial disclosure

This research was supported financially by Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.

Acknowledgment

The authors greatly thanks vice chancellor of research of Babol University of Medical Sciences for approving and supporting this study.

REFERENCES

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics. *CA Cancer J Clin* 2014;**64**:9–29.
2. Zaleska K. miRNA-Therapeutic tool in breast cancer? Where are we now? *Rep Pract Oncol Radiother* 2015;**20**:79–86.
3. Feng JF, Lu L, Zeng P, et al. Serum total oxidant/antioxidant status and trace element levels in breast cancer patients. *Int J Clin Oncol* 2012;**17**:575–83.

4. Montazeri A, Vahdaninia M, Harirchi I, et al. Breast cancer in Iran: need for greater women awareness of warning signs and effective screening methods. *Asia Pac Fam Med* 2008;**7**:6.
5. Manoharan S, Pugalendhi P. Breast cancer: an overview. *Cell Tissue Res* 2010;**10**:2423–32.
6. Babaei Z, Moslemi D, Parsian H, Khafri S, Pouramir M, Mosapour A. Relationship of obesity with serum concentrations of leptin, CRP and IL-6 in breast cancer survivors. *J Egypt Natl Canc Inst* 2015;**27**:223–9, http://dx.doi.org/10.1016/j.jnci.2015.09.001.
7. Franca CAS, Nogueira CR, Ramalho A, Carvalho ACP, Vieira SL, Penna ABRC. Serum levels of selenium in patients with breast cancer before and after treatment of external beam radiotherapy. *Ann Oncol* 2011;**22**:1109–12.
8. Acevedo C, Amaya C, López-Guerra JL. Rare breast tumors: review of the literature. *Rep Pract Oncol Radiother* 2014;**19**:267–74.
9. Murawa P, Murawa D, Adamczyk B, Połom K. Breast cancer: actual methods of treatment and future trends. *Rep Pract Oncol Radiother* 2014;**19**:165–72.
10. Nair CK, Parida DK, Nomura T. Radioprotectors in radiotherapy. *J Radiat Res* 2001;**42**:21–37.
11. Darby SC, Ewertz M, McGale P, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. *N Engl J Med* 2013;**368**:987–98.
12. Shapiro CL, Hardenbergh PH, Gelman R, et al. Cardiac effects of adjuvant doxorubicin and radiation therapy in breast cancer patients. *J Clin Oncol* 1998;**16**:3493–501.
13. Harper JL, Franklin LE, Jenrette JM, Aguero EG. Skin toxicity during breast irradiation: pathophysiology and management. *South Med J* 2004;**97**:989–93.
14. Curtis RE, Boice Jr JD, Stovall M, et al. Risk of leukemia after chemotherapy and radiation treatment for breast cancer. *N Engl J Med* 1992;**326**:1745–51.
15. Algara M, Arenas M, Bayo D.D.P.E., et al. Radiation techniques used in patients with breast cancer: results of a survey in Spain. *Rep Pract Oncol Radiother* 2012;**17**:122–8.
16. Hojan K, Milecki P. Opportunities for rehabilitation of patients with radiation fibrosis syndrome. *Rep Pract Oncol Radiother* 2014;**19**:1–6.
17. Marín A, Martín M, Liñán O, et al. Bystander effects and radiotherapy. *Rep Pract Oncol Radiother* 2015;**20**:12–21.
18. Halimi M, Asghari SM, Sariri R, Moslemi D, Parsian H. Cellular response to ionizing radiation: a microRNA story. *IJMCM* 2012;**1**:178–84.
19. Halimi M, Parsian H, Asghari SM, et al. Clinical translation of human microRNA 21 as a potential biomarker for exposure to ionizing radiation. *Transl Res* 2014;**163**:578–84.
20. Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. *J Nutr Biochem* 2007;**18**:567–79.
21. Sabitha KE, Shyamaladevi CS. Oxidant and antioxidant activity changes in patients with oral cancer and treated with radiotherapy. *Oral Oncol* 1999;**135**:273–7.
22. Borek C. Antioxidants and radiation therapy. *J Nutr* 2004;**134**:3207S–9S.
23. Fraunholz I, Eberlein K, Schopohl B, Bottcher HD, Rodel C. Selenium levels during the course of radiotherapy. *Strahlenther Onkol* 2008;**184**:411–5.
24. Belwalkar G, Badkah D, JaGtap P, Pujari P. Effects of radiation therapy on estrogen and oxidative stress in postmenopausal women with breast cancer. *Int J Pharm Biol Sci* 2012;**3**:B-600–4.
25. Pajovic SB, Pejic S, Kasapovic J, Radojcic MB, Borojevic ND, Radosevic-Jelic LM. Role of superoxide dismutase in individualization of breast cancer radiation therapy protocols. *Arch Oncol* 2003;**11**:191–2.
26. Kasapovic J, Pejic S, Todorovic A, et al. Antioxidant status in breast cancer patients of different ages after radiotherapy. *Arch Biol Sci* 2009;**61**:23–8.
27. Kasapovic J, Pejic S, Stojiljkovic V, et al. Antioxidant status and lipid peroxidation in the blood of breast cancer patients of different ages after chemotherapy with 5-fluorouracil, doxorubicin and cyclophosphamide. *Clin Biochem* 2010;**43**:1287–93.
28. Khoshbin AR, Mohamadabadi F, Vafaeian F, et al. The effect of radiotherapy and chemotherapy on osmotic fragility of red blood cells and plasma levels of malondialdehyde in patients with breast cancer. *Rep Pract Oncol Radiother* 2015;**20**:305–8.
29. Nwozo SO, Solomon O, Abimbola OO, Kikelomo DO. Comparative study of biochemical and nutritional status of breast cancer patients on chemotherapy/radiotherapy in Ibadan. *Am J Cancer* 2013;**2**:51–60.
30. Carlson RW, Allred DC, Anderson BO, et al. Breast cancer. *J Natl Compr Cancer Netw* 2009;**7**:122–92.
31. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal Biochem* 1996;**239**:70–6.
32. Rael LT, Thomas GW, Craun ML, Curtis CG, Bar-Or R, Bar-Or D. Lipid peroxidation and the thiobarbitoric acid assay: standardization of the assay when using saturated and unsaturated fatty acids. *J Biochem Mol Biol* 2004;**37**(6):749–52.
33. Thanoon I, Jadoa AJ, Ahmed KRFA. Oxidant/antioxidant status in serum of breast cancer women treated by surgical interference and chemotherapy. *Iraq J Pharm* 2013;**13**:7–12.
34. EL-Bindarya AA, Yahyab RS, EL-Mezayenc HA, Darwishd H, Eissae MA. Antioxidants status in breast cancer patients under chemotherapy. *Nat Sci* 2013;**11**:1–6.
35. Baranwal M, Singh SK, Nigam A, Singh N, Singh N. Oxidant and antioxidant activity alteration in anaplastic astrocytomas patients following radiation therapy. *JMSCR* 2014;**2**:1194–203.
36. Crohns M, Saarelainen S, Kankaanranta H, Moilanen E, Alho H, Kellokumpu-Lehtinen P. Local and systemic oxidant/antioxidant status before and during lung cancer radiotherapy. *Free Radic Res* 2009;**43**:646–57.
37. Wozniak A, Masiak R, Szpinda M, et al. Oxidative stress markers in prostate cancer patients after HDR brachytherapy combined with external beam radiation. *Oxid Med Cell Longev* 2012, http://dx.doi.org/10.1155/2012/789870.
38. Demirci S, Ozsaran Z, Celik HA, Aras AB, Aydin HH. The interaction between antioxidant status and cervical cancer: a case control study. *Tumori* 2011;**97**:290–5.
39. Schmid SM, Eichholzer M, Bovey F, Myrick ME, Schotzau A, Guth U. Impact of body mass index on compliance and persistence to adjuvant breast cancer therapy. *Breast* 2012;**21**:487–92.
40. Carmichael AR, Bates T. Obesity and breast cancer: a review of the literature. *Breast* 2004;**13**:85–92.
41. Cui Y, Whiteman MK, Flaws JA, Langenberg P, Tkaczuk KH, Bush TL. Body mass and stage of breast cancer at diagnosis. *Int J Cancer* 2002;**98**:279–83.