REPORTS OF PRACTICAL ONCOLOGY AND RADIOTHERAPY XXX (2014) XXX–XXX

Available online at www.sciencedirect.com

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

Original research article

The effect of radiotherapy and chemotherapy on osmotic fragility of red blood cells and plasma levels of malondialdehyde in patients with breast cancer

Ali Reza Khoshbin[a], *Fahimeh Mohamadabadi*[b], *Fatemeh Vafaeian*[b], *Adel Babania*[c], *Saeideh Akbarian*[c], *Reza Khandozi*[d], *Mohamad Ali Sadrebazaz*[d], *Ehsan Hatami*[d], *Hamid Reza Joshaghani*[a,b,c,d,*]

[a] *Biochemistry & Metabolic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran*
[b] *Faculty of Medical Sciences, Golestan University of Medical Science, Gorgan, Iran*
[c] *Student Research Committee, Golestan University of Medical Sciences, Gorgan, Iran*
[d] *Golestan Research of Gastroenterology and Hepatology, Golestan University of Medical Science, Gorgan, Iran*

ARTICLE INFO

Article history:
Received 1 February 2014
Received in revised form
24 July 2014
Accepted 3 November 2014

Keywords:
Breast cancer
Red blood cell
Osmotic fragility
Malondialdehyde

ABSTRACT

Background: Gamma radiation effects on the erythrocyte membrane from three different functional parts, lipid bilayer, cytoskeleton and protein components. When the red cell membrane is exposed to radiation, it loses its integrity and hemoglobin leaks out. In addition, irradiation leads to lipid peroxidation and the products of this process, leading to hemolysis. The aim of the present study was to measure osmotic fragility (OF) of red blood cells and malondialdehyde (MDA) levels as a marker of oxidative injury in breast cancer patients treated with radiation and chemotherapy.

Materials and Methods: The OF test was performed using different concentrations of a salt solution. The measurement of MDA was done with chemical methods.[11] The sampling was taken during three stages of treatment: first sample was taken before starting chemotherapy, the second sample was taken before radiation therapy and the third sample was taken after radiotherapy.

Results: No statistically significant differences between levels of MDA in these three stages of treatment were observed. However, the comparison of mean levels of MDA showed an increase after radiotherapy. The OF rate did not show significant difference ($P > 0.05$) during the stages of treatment.

* *Corresponding author at*: Golestan Research Center of Gastroenterology and Hepatology, Building No 2, 3rd Floor, 10th Azar Alley, 5 Azar Blvd. Gorgan 4917765181, Iran. Tel.: +98 1712340835.
E-mail address: joshaghani@goums.ac.ir (H.R. Joshaghani).

http://dx.doi.org/10.1016/j.rpor.2014.11.002

Conclusion: In a standard treatment program of radiotherapy and chemotherapy lipid peroxidation level and OF do not significantly increase.

1. Background

Breast cancer is a major public health problem in the world, with incidence of more than one million a year, and nearly half of it occurs in North America and Europe.[1] In 2012, 1.7 million new cases of breast cancer were diagnosed worldwide, and in the same year 52,2000 women died from it.[2] The prognosis of this disease depends on the tumor characteristics and quality of treatments. These treatments include systemic and local approaches based on chemotherapy and then hormonal interventions to prevent or delay metastasis.[1] Approximately 5–10% of breast and ovarian cancers are hereditary and 30–50% are autosomal dominant mutations. Deleterious gene mutations in BRCA1 and BRCA2 are responsible for hereditary breast cancer.[3]

Gamma rays used in radiotherapy have different biological effects on the membrane of red blood cells. Free radicals that are produced during body irradiation can cause membrane changes including lipid peroxidation, phospholipid hydrolysis and disulfide bridged formation.[4] Also, these changes in the cell membrane may affect the cytoskeleton. The effect of free radicals on the erythrocyte membrane and cytoskeleton can cause defects and hemolysis in the red blood cells and hemoglobin leakage.[5] For evaluation of osmotic fragility of red blood cells and disease diagnosis related to hemolysis osmotic fragility (OF), a test was performed.[6] Patients with breast cancer under adjuvant treatment suffer from a decrease in hemoglobin concentration that in cancer patients has been related to high levels of fatigue and depression with a decrease in physical activity, quality of life and survival.[7] Based on the previous studies, the importance of this test is to confirm that the membrane property and morphology of red blood cells is abnormal.[6]

The free radicals from radiation have a high affinity to the lipid membrane, which may cause damage to the membrane. These damaged lipid molecules are hardly back to the initial state and cause a gap in the cell and disorder in exchanging important intracellular minerals.[8,9] To evaluate the oxidative damage of erythrocyte, malondialdehyde (MDA) was used as a marker for measuring lipid peroxidation levels.[10] The aim of this study was to evaluate the effect of radiotherapy and chemotherapy on the fragility of red blood cells and plasma MDA levels as a marker of oxidative damage.

2. Materials and methods

This descriptive-analytical study was performed in 2013 on 17 patients with breast cancer recently diagnosed and who had followed surgery.

Fasting venous blood was taken for OF and MDA measurement. OF was evaluated by a method using different concentrations of salt solution and MDA was assayed with the chemical method developed by Satoh.[11] The sampling was taken during three stages of treatment: first sample was taken before starting chemotherapy, the second sample was taken before radiation therapy and the third sample was taken after radiotherapy. All patients received the same drug regimen, including Taxotere (docetaxel), Adriamycin (doxorubicin) and cyclophosphamide in chemotherapy. Concerning radiotherapy, all patients received radiation dose of 54 Gy in the chest wall and supraclavicular regions as a standard protocol delivered by the Phoenix radiotherapy machine. This study was approved by the ethical committee of Golestan University of medical sciences and patients participated on a voluntary and conscious basis.

2.1. *Statistical test*

Statistical analysis was performed using the SPSS software (v.16.0). Mean values and standard deviations were calculated for all parameters. Pearson's correlation was used to correlate the markers of oxidative injury (MDA levels and OF). Comparisons between each stage of treatment were done using a *t*-test.

3. Results

The mean and standard deviation age of the patients was 51.5 ± 1.05 years and the average radiation dose was 4693 ± 219.4 cGy. Plasma level of MDA and erythrocyte OF increased during three stages (Table 1), but no significant statistical difference was observed (Table 2).

Despite of the increasing trend of MDA and OF in each stage compared to the previous stage, no significant difference was found between groups ($P > 0.05$).

4. Discussion

This study evaluated the levels of MDA and OF in patients with breast cancer who had undergone chemotherapy and radiotherapy. The comparison of the mean of MDA levels and OF showed an increasing trend from beginning to end of treatment.

Few studies have been performed on this subject. Some results are in accordance with ours, while some others are contrary to our results. The study performed by Kergonou and colleagues evaluated the effects of gamma radiotherapy on the lipid peroxidation and osmotic fragility of rat erythrocytes. The authors found that MDA levels increased and rat erythrocyte fragility decreased after radiotherapy.[12] In another study performed by Selim and coworkers to assess the effect of radiotherapy on the biophysical characteristics of the

Table 1 Mean, standard deviation, minimum and maximum levels of plasma malondialdehyde (MDA) and osmotic fragility (OF) during three phases of treatment.

	MDA1 (ng/ml)	MDA2 (ng/ml)	MDA3 (ng/ml)	OF1 (g/dl)	OF2 (g/dl)	OF3 (g/dl)
Mean	1.48	1.60	1.88	0.52	0.54	0.56
SD	0.95	1.18	1.26	0.035	0.028	0.022
Minimum	0.50	0.30	0.60	0.50	0.50	0.55
Maximum	4.00	4.80	3.90	0.60	0.60	0.60

Table 2 Comparison of mean levels of malondialdehyde between stages of treatment.

	MDA (ng/ml) SD ± mean	*P* value	OF (g/dl) SD ± mean	*P* value
C1-C2	−0.05 ± 1.52	0.897	−0.025 ± 0.05	0.072
C2-C3	−0.75 ± 1.45	0.190	−0.011 ± 0.033	0.347
C1-C3	−0.085 ± 1.77	0.902	−0.025 ± 0.057	0.227

C1: before chemotherapy; C2: before radiotherapy; C3: after radiotherapy.

erythrocyte membrane, such as the fragility of red blood cells in rats, the results showed that the average osmotic fragility was decreased.[13] These two studies were inconsistent with the present study. The results showed, despite plasma MDA and OF increasing levels during treatment stages, that there was no significant difference between them.

Studies show that increasing the MDA levels from radiotherapy can constitute cross connections with the first amino group of proteins or membrane phospholipids, leading to more strength of erythrocyte membranes. This event could possibly cause resistance of the erythrocyte membrane to hemolysis in creating hypotonic conditions to measure OF.[12] This event might represent no changes in the OF level during the treatment stages in this study. Although for a more accurate conclusion further studies with a larger statistical population are needed. Dolan and coworkers assumed that aerobic exercise training increases RBC production. The results showed that the regular exercise did not have a protective effect against the decline in Hb in patients with breast cancer who were under chemotherapy, but there was a stronger association between Hb and VO_2 peak.[7]

Knopik-Skrock and coworkers evaluated the hemolysis process caused by amphotericin-Bin erythrocytes in women with breast cancer, before and during treatment (surgery, chemotherapy). A significant increase in resistance to amphotericin-B was observed after surgery ($P < 0.01$) that was appropriate with the decrease in the erythrocyte number, hemoglobin and hematocrit. Chemotherapy did not change the resistance of erythrocytes to amphotericin-B. Osmotic fragility of patient erythrocytes did not show any significant differences compared with healthy subjects. This indicates that the resistance of erythrocytes to amphotericin-B was not determined by the membrane's volume and surface area,[14] which is compatible with the present study. Cetin and colleagues assayed plasma antioxidant concentration in patients treated with high dose chemotherapy. They measured zinc, silver, iron in erythrocytes, MDA, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) before high dose chemotherapy (baseline), before stem cell transplantation (day 1) and after stem cell transplantation (days 1, 3 and 6). After high dose chemotherapy, a significant increase was observed in the levels of MDA, GSH-Px and SOD. On the other hand, Copper levels remained the same, while the levels of iron and zinc in erythrocytes were increased. A significant correlation was observed between the levels of MDA, GSH-Px and SOD ($P < 0.05$). The results showed that high dose chemotherapy causes an increase in the oxidative stress and the reactive oxygen species.[15]

Ladas and coworkers considered the fact that many cancer patients take antioxidant nutritional supplements to improve long-term outcomes, but still the efficacy and safety of antioxidants are not known. Their study suggested that the talantioxidant status (measured by total radical antioxidant parameters) decreases during cancer treatment.[16]

Volker and coworkers evaluated the safety and efficacy of EpoetinAlfa in a second randomization of the intense dose-dense arm of the 1284 patients. The primary efficacy end points consisting change in hemoglobin level from baseline to cycle 9 and the percentage of people needed blood transfusion. The results showed that EpoetinAlfa did not decrease in hemoglobin level versus the control group ($P < 0.01$) and statistically significant reduction of the percentage of patients requiring blood transfusion. However, the incidence of thrombotic events increased (7% vs. 3% in the control group). After 62-month treatment follow-up with EpoetinAlfa, no effect was observed on overall survival, relapse-free survival, or intramammary relapse.[17]

5. Conclusion

The levels of MDA and OF at each stage of the treatment showed no significant difference, but its average level increased during the three stages of treatment. The osmotic fragility rate did not show changes in any of the three stages. According to how much the level of lipid peroxidation increases in cancer, radiotherapy can increase it furthermore. Assay of OF in patients with long duration radiotherapy or chemotherapy was recommended.

Conflict of interest

None declared.

Financial disclosure

None declared.

Acknowledgments

We wish to thank the personnel of the radiotherapy department of the Gorgan 5th Azar hospital and Kavosh medical laboratory personnel, specially Mr. Hedayat-Mofidi. This study was supported by deputy head for research and technology of the Golestan University of Medical Sciences.

REFERENCES

1. Bougnoux Ph, Hajjaji N, Maheo K, Couet Ch, Chevalier S. Fatty acids and breast cancer: sensitization to treatments and prevention of metastatic re-growth. *Prog Lipid Res* 2010;**49**:76–86.
2. Ferlay J, Soerjomataram I, Ervik M, et al. *GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 Internet*. Lyon, France: International Agency for Research on Cancer; 2013. Available from http://globocan.iarc.fr/
3. Jalkh N, Nassar-Slaba J, Chouery E, et al. Prevalance of BRCA1 and BRCA2 mutations in familial breast cancer patients in Lebanon. *Hereditary Cancer Clin Pract* 2012;**10**:1–7.
4. Lee SW, Ducoff HS. The effects of ionizing radiation on avian erythrocytes. *Radiat Res* 1994;**137**(1):104–10.
5. Schön W, Ziegler C, Gärtner H, Kraft G. Heavy ion induced membrane damage: hemolysis of erythrocytes and changes in erythrocyte membrane fluidity. *Radiat Environ Biophys* 1994;**33**(3):233–41.
6. Wintrobe MM, Lee RH, Boggs DR, Bithell TC, Athens JW, Foerster J. *Clinical haematology*. 7th edition Philadelphia: Lea and Febiger; 1976. p. 774–5.
7. Dolan BL, Gelmon K, Courneya SK, et al. Hemoglobin and aerobic fitness changes with supervised exercise training in breast cancer patients receiving chemotherapy. *Cancer Epidemiol Biomarkers Prev* 2010;**19**:2826–32.
8. Marnett LJ. Lipid peroxidation—DNA damage by malondialdehyde. *Mutat Res/Fundamental Mol Mech Mutagen* 1999;**424**(1):83–95.
9. Pajović SB, Saičić ZS, Pejić S, Kasapović J, Stojiljković V, Kanazir DT. Antioxidative biomarkers and cancerogenesis. *Jugoslovenska medicinska biohemija* 2006;**25**: 397–402.
10. Gönenç A, Özkan Y, Torun M, Şimşek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. *J Clin Pharm Ther* 2001;**26**(2):141–4.
11. Kei S. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* 1978;**90**(1):37–43.
12. Kergonou J, Thiriot C, Braquet M, Ducousso R, Rocquet G. Influence of whole-body (-irradiation upon rat erythrocyte: lipid peroxidation and osmotic fragility. *Biochimie* 1986;**68**(2):311–8.
13. Selim NS, Desouky O, Ali SM, Ibrahim I, Ashry HA. Effect of gamma radiation on some biophysical properties of red blood cell membrane. *Romanian J Biophys* 2009;**19**:171–85.
14. Skrocka K, Bielawski A, Kopczyński J, et al. The susceptibility to amphotericin B-induced hemolysis of erythrocytes from women with breast cancer before and during therapy. *Comp Clin Pathol* 2006;**15**:181–7.
15. Cetin T, Arpaci F, Yilmaz MI, et al. Oxidative stress in patients undergoing high-dose chemotherapy plus peripheral blood stem cell transplantation. *Biol Trace Element Res* 2004;**97**(3):237–47.
16. Ladas EJ, Jacobson JS, Kennedy DD, Teel K, Fleischauer A, Kelly KM. Antioxidants and cancer therapy: a systematic review. *J Clin Oncol* 2004;**22**(3):517–28.
17. Moebus V, Jackisch C, Schneeweiss A, et al. Adding epoetin alfa to intense dose-dense adjuvant chemotherapy for breast cancer: randomized clinical trial. *J Natl Cancer Inst* 2013;**105**(14):1018–26.