

# CBC and Total Antioxidant Capacity in Breast Cancer Patients After Radiotherapy

Fahad Riyadh Khudhair<sup>1</sup>, Anfal Ali Shakir<sup>2</sup>, Hayder Shkhair Obayes Al-Janabi<sup>3</sup>

<sup>1, 2, 3</sup>Department of Biotechnology/ College of Biotechnology/ Al-Qasim Green University/Iraq

Email: [fahd4603@gmail.com](mailto:fahd4603@gmail.com)

Email: [anfal@biotech.uoqasim.edu.iq](mailto:anfal@biotech.uoqasim.edu.iq)

## Abstract

**Background:** The term "cancer" refers to disorders in which aberrant cells proliferate unchecked and can infect other tissues. The lymphatic and vascular systems of the body can allow cancer cells to spread to other areas of the body. The most frequent method of irradiation used in radiotherapy to treat cancer patients is the use of an external beam of photons or electrons. X-ray or Y-ray photons are thought of as energy bundles that deposit dosage as they go through material. **Objectives:** 1-How radiation therapy affects the blood system and some blood parameters 2- The effect of radiotherapy on antioxidant capacity in serum level **Methods:** 90 women participated in this study, and they were split into three primary groups: Group I: 30 individuals with stage II-stage III invasive breast cancer; Group II: The same 30 patients After Radiotherapy, Group III: 30 healthy women as a control group. After surgery and chemotherapy, all patients in the study got adjuvant radiation. For physiological study Hematological analyzer device is used for complete blood count (CBC) to measure White Blood Cells (WBC) and its differential Granulocyte (GRA), Monocyte (MON), Lymphocyte (LYM) and Red Blood Cells (RBC), Platelet (PLT), Hematocrit (HCT), Hemoglobin (HGB). For biochemical study the enzyme-linked immunosorbent test was used to quantify Total Antioxidant Capacity (T-AOC). **Results:** There was a significant decrease occurred in the percent (WBC) between Group I patients and Group III control, while its differential shows (LYM) significantly increases and (GRA) significantly decreases. Also (HGB) significantly increase and no change to other parameters. A significant decrease occurred in the percent of (WBC) between Group I patients and Group II patients After Radiotherapy and it's differential shows significant decrease in (LYM) and significant increase in (GRA) while no significant changes in other parameters.(T-AOC) shows significant increase between Group I and Group II After radiotherapy while no change between Group I and Group III. **Conclusion:** There was an effect of radiotherapy on (WBC) and its differential and on (T-AOC).

**Keywords:** Radiotherapy, Breast cancer, Patients, CBC, Antioxidant.

## 1. Introduction

Breast cancer is the most common cancer among women worldwide, and the leading cause of mortality in both developing and developed countries (Al-Saigh et al., 2020). Breast cancer has become a big concern to women in Iraq, and it is the most common type of cancer, accounting for 34.4 % of all cancers in the country (Iraqi Cancer Board, 2014). During the last few decades, the use of radiotherapy as a central aspect of curative treatment for a variety of cancers has progressed (Abulkassim et al., 2014). Acute and chronic side effects might occur as a result of radiotherapy. Blood cell counts can change, mainly in the form of a drop in cell numbers (Shahid, 2016). Leucocytes, erythrocytes, and platelets are all blood cells that can interact with carcinomas in one way or another (Wersal et al., 2019). The severity of these side effects is determined by a number of parameters, including the radiation dose, delivery rate, and treatment duration, type of radiation, exposure site, and patient age (Abulkassim et al., 2014). A complete blood count is a required study for all cancer patients prior to surgery, chemotherapy, or radiotherapy (Shrivastava et al., 2017). In healthy

people, blood contains a variety of cells in proper amounts. Because any type of severe disease or abnormality has a direct impact on blood parameters, it's important to track changes in hematological parameters in breast cancer patients at regular intervals during treatment, such as chemotherapy and radiotherapy (Shrivastava et al., 2017). A complete blood count (CBC) is a blood test that determines the types and quantities of red blood cells, white blood cells, and platelets in the body. The CBC allows doctors to check for any symptoms that a patient may be experiencing, such as weakness, exhaustion, or bruises. It can also aid in the diagnosis of other illnesses (Ali, 2014). Anemia is a common complication in cancer patients, and it can be caused by a variety of factors including bleeding, nutritional inadequacies, bone marrow destruction, tumor infiltration in the bone marrow, and the malignant process itself. Tumor necrosis factor (TNF- $\alpha$ ) and interleukin-1 (IL-1) are inflammatory cytokines linked to tumor genesis that can stop erythrocytic progenitors from proliferating (varlotto and Stevenson, 2005). Antioxidants are substances that can aid in preventing the chemical chain's damaging interactions with oxygen and/or nitric oxide. Oxidation reactions are what are known as this process. Antioxidants work by reacting with free

radicals that interact with certain molecules to prevent oxidation. Additionally, antioxidants may strengthen the immune system, which lowers the risk of infection and cancer development. Antioxidants assist in balancing the healthy oxidant generation and frequently function as cell signaling those harms from oxidative stress (Mahmood and Khalaf, 2018).

### 1.2 Aims of the study.

Study and investigate the effect of radiation dose on different stages of breast cancer (physiological, Biochemical).

## 2. Materials and Methods

### 2.1 Sampling and Study design

Blood samples have been collected from 30 patients with invasive breast carcinoma in Babil Oncology Center/Babil Iraq from a period of December 2021 to April 2022. This case-control study including 90 samples divided into three groups:

Group I: 30 Patients who suffered stage II-III primary invasive breast cancer.

These patients have been diagnosed by physician with all clinical examination and clinical evaluation preformed including physical examination, chest X-ray, blood tests, abdominal ultrasound, breast mammography and ultrasound. Also, Immunohistochemistry assessed pretreatment estrogen, progesterone receptors and HER 2 status and all patients treated with chemotherapy and had a surgery before treating with radiotherapy. Also, we considered age, tumor location in this study.

Group II: The same 30 patients after completing the total radiotherapy dose. Each patient received radiotherapy by linear accelerator. The total dose given (40.5 Gry) dividing on to (15 fraction) the daily

dose were (2.7 Gry) of locally x-ray radiation treatment for a five days a week.

Group III: 30 healthy women with matched age as a control group.

### 2.2 Laboratory analysis

The volume of 3ml of peripheral blood samples were collected by disposable syringes and divided into two parts : 1 ml in EDTA tube which used for our physiological study (CBC) and 2 ml in gel tube left for 20 minutes , then centrifuged for 5 minutes at 3000 RPM, Serum was transferred into 2ml Eppendorf tubes and stored at -40 °C in deep freezer which used later for Biochemical study (T-AOC). We used (Humacount 30TS, Germany) device for complete blood count to measure White Blood Cells(WBC) and its differential Granulocyte (GRA), Monocyte (MON), Lymphocyte (LYM) and Red Blood Cells (RBC), Platelet (PLT), Hematocrit (HCT), Hemoglobin (HGB). We also measured (T-AOC) levels by Enzyme-Linked Immunosorbent Assay (ELISA) in accordance with the directions provided by the manufacturer (BT LAB, China). This work done in AL-qassim green university.

### 2.3 Statistical analysis

The SPSS statistics program version 18 (IBM, Armonk, NY, United States of America) was used for the statistical analyses, and outcomes with P values ( $\leq 0.05$ ) were deemed statistically significant.

## 3. 3. Results and Discussion

### 3.1 Patients' clinical characteristics

Table 1 summarizes the clinical features of the study subjects.

**Table (1): Clinical characteristics of the participants.**

Patients No.		Characteristics
30	Female	Gender
30	(31-73)	Age
20 10	Right Left	Tumor localization
16 14	II III	Tumor stage
30 0	Invasive ductal other	Pathology
21 9	+ -	ER status
22 8	+ -	PR status
8 22	+ -	HER 2 immunohistochemistry
23 7	Mastectomy lumpectomy	Surgery
30 0	Yes No	Chemotherapy
30 0	Yes No	Radiotherapy

### 3.2 Physiological Study

#### 3.2.1 Complete Blood count Before Radiotherapy

As shown in Table (2), in this study we had compared patients of Breast Cancer group who had chemotherapy and surgery treatment with a healthy control group with the same ages. The result was there where a significant decrease in WBC and its differential LYM and GRA at  $p \leq 0.01$  with no significant change in MON, while HGB will significantly decrease at  $p \leq 0.01$  and no significant change in other parameters.

**Table (2): CBC in Control and Breast Cancer patients.**

Parameters	Groups		P-value
	Control	Patient	
	Mean±S.E		
WBC ( $10^3/\mu\text{l}$ )	8.29±0.5	5.91±1.1	$\leq 0.0001^{**}$
LYM (%)	21.40±4.4	30.12±2.3	$\leq 0.0001^{**}$
MON (%)	5.53±0.6	5.79±0.3	0.452
GRA (%)	73.12±15.6	64.01±16.3	$\leq 0.0001^{**}$
RBC ( $10^6/\mu\text{l}$ )	4.46±0.9	4.26±0.7	0.171
HGB(g/dl)	10.20±1.2	11.52±1.8	0.003**
HCT (%)	34.78±7.1	36.30±4.6	0.201
PLT ( $10^3/\mu\text{l}$ )	266.30±17.8	249.10±28.9	0.338

\*\* refer on significant difference at  $p \leq 0.01$ .

A routine test called the complete blood picture is frequently used by clinicians to support the working diagnosis of a number of diseases, including anemia, acute infections, hemorrhagic states, allergic disorders, cancers, and immune disorders, as well as for pre-operative assessments and health screenings. Cancer patients frequently experience anemia, and chemotherapy makes it more common. Chemotherapy also raises the likelihood of needing transfusions. Cancer patients who are anemic have a lower chance of surviving and experience more fatigue. Cancer stimulates the production of inflammatory cytokines, which inhibits the development of erythropoiesis and erythropoietin (EPO) (Spivak et al., 2009). Chemotherapy side effects during the adjuvant chemotherapy procedure include low blood counts (Miller and Heilmann, 1988). Absolute lymphocytes count significantly decreased during chemotherapy, which is one of the unfavorable effects of chemotherapy on breast cancer patients (Ali, 2014). It might be caused by elevated levels of pro-inflammatory cytokines including IL-1, IL-6, TNF- $\alpha$ , and INF- $\gamma$ , which cause iron retention in the liver, gastrointestinal tract, and reticulo-endothelial system and block the production of erythroid precursors (Moraes et al., 2000).

In this study we had found that WBC and its differential LYM and GRA and also HGB had significantly decrease at  $p \leq 0.01$  which agree with other Iraqi studies (Ali, 2014; Alsaadi and Younus, 2019), also agree with other study in India (Shrivastava et al., 2017). While other parameters RBC, PLT, HCT Shows no significant change which disagree with studies (Ali, 2014; Ali et al., 2018; Alsaadi and Younus, 2019) and other study in India (Shrivastava et al., 2017).

### 3.2.2 Complete Blood count After Radiotherapy

As shown in Table (3), in this study we had compared patients of Breast Cancer group who had chemotherapy and surgery treatment with the same patients After Radiotherapy healthy. The result was there where a significant decrease in WBC and its differential LYM, with significant increase in GRA at  $p \leq 0.01$  with no significant change in MON, while there is no significant change in other parameters.

Table (3): CBC in patients before and after radiotherapy

Groups Parameters	Before	After	P-value
	Radiotherapy	Radiotherapy	
	Mean±S.E		
WBC ( $10^3/\mu\text{l}$ )	5.91±1.1	4.39±0.3	0.003**
LYM (%)	30.12±2.3	22.40±0.8	$\leq 0.0001$ **
MON (%)	5.79±0.3	6.10±0.5	0.584
GRA (%)	64.01±16.3	71.14±3.9	$\leq 0.0001$ **
RBC ( $10^6/\mu\text{l}$ )	4.26±0.7	4.23±0.1	0.685
HGB(g/dl)	11.52±1.8	11.64±0.2	0.955
HCT (%)	36.30±4.6	36.25±0.5	0.201
PLT ( $10^3/\mu\text{l}$ )	249.10±28.9	227.34±13.9	0.228

\*\* refer on significant difference at  $p \leq 0.01$ .

Any type of radiation, including x and gamma rays as well as charged or uncharged particles, is absorbed in biological material and interacts with DNA directly

to cause biological change that may be visible in biological fluids such as blood (Ramaswamy, 2008) Radiation traditionally targets the hematological, gastrointestinal, neurological, and circulatory systems (Donnelly et al., 2010 ) this study shows an agreement in WBC change and its differential in Iraqi studies (Saydoka and Zaki, 2013 ; Abulkassim et al., 2014) and other studies (Shrivastava et al., 2017; Wersal et al., 2019) while it disagree with other parameters shown in Table (4-3).

### 3.3 Biochemical study

#### 3.3.1 Serum levels of Antioxidant capacity in patients with breast cancer

By ELIZA technique we measured T-AOC concentration in serum of breast cancer patients and healthy women. First we compared the results between breast cancer patients before treated with radiotherapy with healthy women as a control group as it's summarized in table (4). The Mean± S.E of T-AOC was  $16.83 \pm 2.3$  in healthy control group while it was  $18.07 \pm 1.6$  in breast cancer patients before radiotherapy .the statistical analysis shows no significant change in serum level T-AOC between healthy control group and breast cancer patients at p-value ( $p \leq 0.01$ ). After radiotherapy the Mean± S.E of T-AOC was  $25.74 \pm 2.2$  as it summarized in table (5). That means the statistical analysis shows a significant increase between breast cancer patients before and after radiotherapy treatment at p-value ( $p \leq 0.01$ ). Figure (1) shows the mean difference between the three groups.

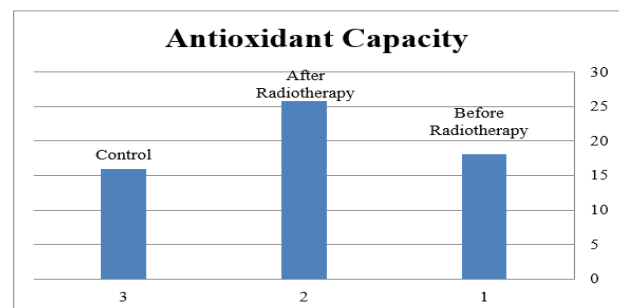


Figure (1): A bar graph showing the average serum levels of T-AOC (U/mL) in breast cancer patients before and after radiation treatment in comparison to controls.

Table (4): Antioxidant capacity concentration in control and breast cancer patients.

Groups Parameters	Control	Patient	P-value
	Mean± S.E		
T-AOC	$16.83 \pm 2.3$	$18.07 \pm 1.6$	0.673

Table (5): Antioxidant capacity concentration before and after Radiotherapy of Breast Cancer Patients.

Groups Parameters	After	After	P-value
	Radiotherapy	Radiotherapy	
	Mean± S. E		
T-AOC	$18.07 \pm 1.6$	$25.74 \pm 2.2$	0.007**

Through a number of enzyme systems, including catalase, glutathione peroxidase, and superoxide dismutase, antioxidants defend healthy cells against radiation damage. Additionally, non-enzymatic

systems (such selenium, glutathione, and tocopherol) that scavenge free radicals might be beneficial for normal cells. According to some research, radiation may result in people with breast cancer having lower levels of the vitamins A, E, C, and selenium (Arjmandi et al., 2016).

In this study, T-AOC shows a significant increase after radiotherapy which match an agreement with (Arjmandi et al., 2016), and disagreement with (Mahmood and Khalaf, 2018).

#### 4. Conclusion

There was an effect of radiotherapy on (WBC) and its differential and on (T-AOC).

#### References

Abulkassim, R. A., Ismael, D. K., Numan, N. A., Al-Shawi, N. N., & Hussain, S. A. (2014). The Effect of Radiotherapy on Oxidative Stress, Biochemical and Hematological Parameters in Women with Breast Cancer. *Al-Mustansiriyah Journal of Pharmaceutical Sciences (AJPS)*, 14(2), 1-9.

Ali, L. O. (2014). Study effect of breast cancer on some hematological and biochemical parameters in Babylon Province, Iraq. *IOSR (IOSR-JPBS)*, 9(3), 20-24.

Alsaadi, J. H. H., & Younus, B. M. (2009). Study of Some Biochemical and Blood Parameters as Screening Markers for Breast Cancer Patients before Adjuvant Therapy in Thi Qar Government-Southern Iraq.

Al-Saigh, T. H., Al-bayati, S. A., Abdul-mawjood, S. A., & Ahmed, F. A. (2020). Breast Cancer in Mosul: A Survival Analysis. *Al-Mustansiriyah Journal for Pharmaceutical Sciences*, 20(2).

Arjmandi, M. K., Moslemi, D., Zarrini, A. S., Gorji, M. E., Mosapour, A., Haghghighi, A., ... & Parsian, H. (2016). Pre and post radiotherapy serum oxidant/antioxidant status in breast cancer patients: impact of age, BMI and clinical stage of the disease. *Reports of Practical Oncology and Radiotherapy*, 21(3), 141-148.

Donnelly, E. H., Nemhauser, J. B., Smith, J. M., Kazzi, Z. N., Farfan, E. B., Chang, A. S., & Naeem, S. F. (2010). Acute radiation syndrome: assessment and management. *Southern medical journal*, 103(6), 541.

Iraqi Cancer Board: Iraqi Cancer Registry. Ministry of Health, Baghdad, Iraq. 2014.

Mahmood, A. R., Abdullah, M. R., & Khalaf, H. S. (2018). Estimation of some trace elements and antioxidant status in breast cancer patients undergoing radiotherapy. *Journal of Global Pharma Technology*, 10, 213-217.

Miller, B., & Heilmann, L. (1988). Hemorheologic variables in breast cancer patients at the time of diagnosis and during treatment. *Cancer*, 62(2), 350-354.

Moraes, S. P. D., Cunha, A., Reis Neto, J. A. D., Barbosa, H., Roncolato, C. A. P., & Duarte, R. F. (2000). Modelo experimental de tumor de Walker. *Acta Cirúrgica Brasileira*, 15, 237-242.

Ramaswamy, G. (2008). *Washington manual of oncology*.

Saydoka, K. M., & Zaki, S. M. (2013). The effect of radiotherapy treatment on some blood elements in patient with some types of cancer. *Tikrit Medical Journal*, 19(1).

Shahid, S. (2016). Review of hematological indices of cancer patients receiving combined chemotherapy & radiotherapy or receiving radiotherapy alone. *Critical reviews in oncology/hematology*, 105, 145-155.

Shrivastava, S., Singh, N., Nigam, A. K., Chandel, S. S., Shrivastava, R., & Kumar, S. (2017). Comparative study of hematological parameters along with effect of chemotherapy and radiotherapy in different stages of breast cancer. *Int J Res Med Sci*, 5(1), 311-315.

Spivak, J. L., Gascón, P., & Ludwig, H. (2009). Anemia management in oncology and hematology. *The oncologist*, 14(S1), 43-56.

Varlotto J, Stevenson MA. Anemia, tumor hypoxemia and the cancer patient. *Int J Radiat Oncol Biol Phys* 2005; 63: 25-36.

Wersal, C., Keller, A., Weiss, C., Giordano, F. A., Abo-Madyan, Y., Tuschy, B., ... & Sperk, E. (2019). Long-term changes in blood counts after intraoperative radiotherapy for breast cancer—single center experience and review of the literature. *Translational Cancer Research*, 8(5).