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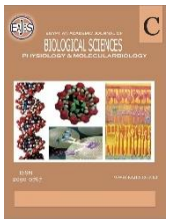

Evaluation of Some Iron Status Markers and Blood Parameters in Women with Breast Cancer

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ABSTRACT

Breast cancer represents the most commonly detected form of cancer in women and stands as the primary contributor to cancer-related mortality among women on a global scale. This study was designed to evaluate the alterations in concentrations of some blood biomarkers implicated in the regulation of iron balance in Iraqi women with breast cancer and to explore the multifaceted role that these indicators play in the progression of this disease. The current investigation comprised a cohort of 55 females diagnosed with breast cancer, with a control group of 45 healthy participants. The levels of iron status markers that include hepcidin, ferritin, and ferroportin were estimated in the serum of breast cancer patients, as well as the values of some hematological parameters such as erythrocytes (RBCs), hemoglobin (Hb), hematocrit (HCT), leukocytes (WBCs), and thrombocytes (platelets). The results of the present study indicated that there were significant differences ($p<0.05$) in all indicators studied in the serum of breast cancer patients in comparison with the control group. Thus, these results may support the evidence about the potential role of these markers in breast cancer disease.

INTRODUCTION

Cancer is a pathological state distinguished by the uncontrolled development and proliferation of cells, wherein these cells have evaded the typical regulatory mechanisms of cellular growth inside the body and acquired the capability to undergo continuous division (Sung *et al.*, 2020).

Breast cancer stands as the most commonly detected form of cancer among women on a global scale, with a staggering 2 million fresh instances reported in 2018. This figure represents roughly a quarter of all newly diagnosed cancer cases in the female population. Breast cancer has the position of being the primary contributor to mortality resulting from cancer in women on a global scale, projecting an estimated 626,700 fatalities in the year 2018 (ACS, 2018).The regulation of iron metabolism is tightly controlled by hepcidin, a peptide generated from the liver. The mechanism of action involves the interaction with ferroportin, a transmembrane protein that is involved in the outflow of iron from the body's iron reserves (Ward and Kaplan, 2012). Besides, the ferritin level serves as a diagnostic indicator for iron-deficiency anemia and as a biomarker in many inflammatory and infectious diseases (Shukla *et al.*, 2023).

There are few studies on the relationship of the subject under study in this research to breast cancer in Najaf governorate. The objective of this study is to evaluate the changes in the concentrations of specific blood biomarkers involved in iron regulation (hepcidin, ferritin, and ferroportin), alongside hematological indicators (erythrocytes, hemoglobin, packed cell volume, leukocytes, and platelets), in females diagnosed with breast cancer, as well as assess the prognostic role of these biomarkers in the early diagnosis of breast cancer.

MATERIALS AND METHODS

This study was conducted, and all of the samples used were obtained from female patients who were admitted to the Al-Furat Al-Awsat Center for Tumors in Al-Najaf City for checking and treatment of breast cancer between 1/2/2023 and 1/5/2023.

Experimental Design:

Patients and Volunteers:

The present investigation exclusively enrolled female patients from Iraq who had been diagnosed with breast cancer and whose diagnoses were confirmed by histopathology reports. This study investigated fifty-five (55) breast cancer patients and forty-five (45) healthy participants. In addition, the participants were appropriately informed about the objectives of the study and gave their informed consent. The project received approval from the scientific ethical committee. All patients underwent comprehensive examinations and received diagnoses from specialized specialists, and the histopathological type of the disease was determined.

Study Groups:

This study included only female breast cancer patients, who were divided into subgroups. The specimens were acquired from individuals who were in good health and had no prior records of chronic or acute illnesses. The ages of the individuals who were in good health in the present investigation were equivalent to the ages of the patients.

Breast Cancer Diagnosis:

Doctors use various tests as part of a physical examination to confirm the presence and severity of breast cancer in order to make a diagnosis. Therefore, the approved medical reports in this study are based on breast examination techniques such as chest x-ray, ultrasound, mammogram, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET) scan, bone scan, and surgical biopsy (ACS, 2022).

Collection of Blood Samples:

To conduct biochemical and hematological analyses, a volume of 5 ml of blood was collected from the cubital vein of both resting patients and control group. The blood collection was performed using a disposable syringe. A volume of 1 ml from each blood sample was transferred into an ethylenediaminetetraacetic acid (EDTA) tube in order to quantify specific hematological parameters.

In order to measure the levels of hepcidin, ferritin, and ferroportin in serum, a volume of four milliliters of blood was aliquoted into glass tubes without the addition of any anticoagulant and afterwards incubated at room temperature for a duration of one hour. Following the process of clotting, the serum samples were separated using centrifugation and subsequently transferred into Eppendorf tubes using a micropipette. These samples were then maintained under freezing conditions at a temperature of -20 °C until they were subjected to analysis (Theml *et al.*, 2004).

Estimation of Iron Status Markers:

Using an enzyme-linked immunosorbent assay (ELISA), the serum hepcidin, ferritin, and ferroportin levels were determined. These kits facilitate the precise measurement of human hepcidin levels. The experiments were conducted in accordance with the guidelines provided by the manufacturers.

Estimation of Hematological Parameters:

The hematological parameters were determined using the usual automated

approach for the complete blood count (CBC). The blood indices assessed in this study using a hematology analyzer comprised the quantification of erythrocytes (RBCs), leukocytes (WBCs), and thrombocytes (platelets), along with the determination of hemoglobin (Hb) and hematocrit (HCT) levels.

The Statistical Analysis:

The statistical analyses were conducted utilizing the statistical package for social sciences (SPSS, version 23). The independent t-test was employed to conduct a comparative analysis among multiple groups. The statistical analysis employed the mean±standard deviation. A significance level of less than <0.05 was deemed appropriate for all statistical tests.

RESULTS AND DISCUSSION

The results of this study demonstrate statistically significant increases ($p<0.05$) in hepcidin and ferritin levels in the serum of individuals diagnosed with breast cancer as compared to healthy individuals. On the other hand, the results showed a statistically significant reduction ($p<0.05$) in the concentration of ferroportin in the serum of individuals diagnosed with breast cancer as compared to healthy individuals,Table (1).

Table 1: Iron status markers in serum of breast cancer patients and control groups.

Parameter	Breast cancer patients	Healthy group
Hepcidin (pg/ml)	659.95±165.05*	536.24±112.55
Ferritin (ng/ml)	387.18±91.12*	50.12±17.74
Ferroportin(ng/ml)	0.95±0.27*	1.37±0.22

- Values are means ± SD. Independent t-test.
- (*) =Significant differences exist at the $p<0.05$.

With regard to hematological parameters, the findings indicate significant decreases ($p<0.05$) in the overall count of RBCs, WBCs, and platelets, in addition to significant decreases ($p<0.05$) in the levels of Hb and HCT. Table (2). In terms of hematological criteria, the results show that the total number of RBCs, WBCs, and platelets, as well as the levels of Hb and HCT, have decreased significantly ($p<0.05$), Table (2).

Table 2: Hematological criteria in serum of breast cancer patients and control groups.

Parameter	Breast cancer patients	Healthy group
RBCs ($10^9/mm^3$)	4.01±0.75*	4.47±0.62
Hb (g/dl)	11.20±1.58*	12.67±1.19
HCT (%)	33.34±4.58*	40.31±3.17
WBCs ($10^3/mm^3$)	5.80±3.06	6.94±1.70
PLT ($10^3/mm^3$)	116.57±9.70	147.15±15.57

- Values are means ± SD. Independent t-test.
- (*) =Significant differences exist at the $p<0.05$.

DISCUSSION

Iron Status Markers:

The results of this study revealed a significant elevation in hepcidin levels and a significant decrease in ferroportin in individuals diagnosed with breast cancer, compared to those who are healthy subjects. The findings are in line with the results of other investigations (Orlandi *et al.*, 2014; Pan *et al.*, 2016; Ali *et al.*, 2020).

Vertebrates maintain iron concentration and body iron content through

hepcidin-regulatory peptide interaction with ferroportin. Hepcidin levels rise as a result of infection and inflammation., limiting iron availability to invading microbes or causing iron restriction or deficiency (Nemeth and Ganz, 2021).The iron metabolism is tightly controlled by hepcidin, which exerts its effect by interacting with ferroportin (Ali *et al.,* 2020). Hepcidin regulates iron outflow from cells through two mechanisms: occlusion of the open-outward conformation of ferroportin (Aschemeyer *et al.,* 2010), and endocytosis and degradation of ferroportin (Nemeth *et al.,* 2004). Hepcidin expression and regulation in tumor cells differ significantly from non-tumorous cells, affecting iron transport and iron sequestration. Hepcidin manipulation is of great interest to scientists (Ganz and Nemeth, 2012). Bone morphogenetic proteins (BMPs) are key stimulators of hepcidin expression in tumor tissues, particularly in breast cancer, and are also linked to increased hepcidin expression (Ciniselli *et al.,* 2015).

However, hepcidin production in breast cancer is not solely derived from cancer tissue, but also from the liver, which is linked to increased BMP6 expression (Zhang *et al.,* 2014). Also, overexpressed BMP7 may be linked to hepcidin overexpression in early breast cancer and is associated with cancer metastasis (Pauk *et al.,* 2015). BMPs, specifically BMP6, play a critical role in breast cancer cell hepcidin production, and their presence in breast cancer spheroids promotes hepcidin via fibroblast-dependent secretion of IL-6 (Blanchette-Farra *et al.,* 2018).

Cytokines augment hepatic hepcidin production, which inhibits iron absorption in the intestines and facilitates iron storage by macrophages, thereby limiting iron recycling (Lamy *et al.,*2014). IL-6 levels are frequently elevated in cancer and are linked to hepcidin levels, particularly in established diseases (Egler *et al.,* 2008). Studies on breast cancer patients with metastasis confirm the significant relationship between serum IL-6 and serum hepcidin (Shao *et al.,* 2017).

The study reveals higher serum ferritin levels in breast cancer patients, consistent with previous clinical findings indicating a positive relationship between elevated ferritin levels and disease prevalence (Orlandi *et al.,* 2014; Pan *et al.,* 2016; Petekkaya *et al.,* 2017).

Recent studies have reported high serum levels of ferritin in patients with metastatic breast cancer (Fadavi *et al.,* 2021; George *et al.,* 2021). In contrast, Holle *et al.* (2021) found that elevated iron stores do not strongly correlate with breast cancer risk, despite the presence of circulating iron, ferritin, and transferrin saturation.

In fact, the increase in ferritin levels in breast cancer tissue and systemic levels is linked to the progression of the disease (Jacobs *et al.,* 1976). Breast cancer patients often experience elevated ferritin levels, possibly due to excess iron stores, inflammation, or both (Macciò *et al.,* 2015).Infiltrating macrophages in breast tumors can release ferritin, which triggers inflammation and is linked to tumorigenesis through an inflammatory effector mechanism (Alkhateeb *et al.,* 2013). In addition, the rise in ferritin and iron levels in breast cancer is linked to an increase in inflammatory markers like C-reactive protein (Mannello *et al.,* 2010).

According to Alkhateeb *et al.* (2013), elevated ferritin levels in breast cancer patient's blood and tumor tissues are linked to poor prognosis and advanced histological grade. In addition, Orlandi *et al.* (2014) found serum hepcidin and ferritin light chain to be effective predictors of cancer of the breast, but weaker indicators of benign breast disease.

Hematological Criteria:

The results of the current investigation have confirmed a notable reduction in some hematological parameters among individuals diagnosed with breast cancer, specifically erythrocyte count, hemoglobin levels (Hb), hematocrit levels (HCT), leukocyte count, and thrombocyte count, in comparison to the control group.

Complete blood count (CBC) criteria are indispensable for clinical decision-making, particularly in the diagnosis of

anemia and other blood disorders (Ahmed *et al.*, 2020). The test could be repeated regularly during treatment as various cancer drugs can affect bone marrow blood-forming cells (ACS, 2021).

According to Khunger *et al.* (2018), the CBC criteria serve as inflammatory indicators that might point to inflammation in tumor tissues. Also, CBC-derived inflammation biomarkers have been utilized as prognostic factors for different malignancies (Shoji *et al.*, 2020).

Anemia is a condition characterized by reduced RBCs number, leading to decreased values of Hb and HCT (Pirker *et al.*, 2003). Anemia caused by cancer is characterized by a reduction in erythropoiesis., resulting from factors such as malnutrition, poor intestinal absorption, chronic inflammation, aging, and advanced cancer stages (Gilreath *et al.*, 2014). Hypoxia can result from anemia and low hemoglobin levels, thereby expediting tumor growth and increasing cancerous cell resistance to both chemotherapy and radiotherapy by the development of multidrug resistance (Milane *et al.*, 2011; Li *et al.*, 2016).

Hemoglobin, a crucial CBC measure, indicates anemia in patients, with women with breast cancer exhibiting significantly lower levels compared to healthy individuals (Ali *et al.*, 2020; Abulkassim and Ismael, 2014).

The results of our study have demonstrated a significant reduction in the number of leukocytes in breast cancer patients. This result contradicts the findings of Ali *et al.* (2020), who discovered an increase in white blood cell count. According to Abulkassim and Ismael (2014), radiotherapy and chemotherapy lead to a decrease in leukocyte numbers and platelets in breast cancer patients. Therefore, these changes in these parameters must be taken into account when treating these patients.

The tumor-induced systemic inflammatory response results in changes in peripheral blood white blood cells (Guthrie *et al.*, 2013). The study found a significant positive correlation between leucocyte and platelet count and survival at a significant level (Gislason and Nõu, 1985). Moreover, dysfunctional platelets and thrombotic conditions are significant indicators of the progression of cancer (Gay and Felding-Habermann, 2011).

CONCLUSION

Iron status markers including hepcidin, ferritin, ferroportin, and hematological criteria including RBCs, hemoglobin, hematocrit, WBCs, and platelets are altered in women with breast cancer compared to healthy individuals. Therefore, the study reveals inexpensive and easy-to-measure tests that can aid in the early detection of breast cancer or further development during treatment.

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