Evaluation of Some Diagnostic Indicators of Breast Cancer Patients in Baqubah City

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Abstract

To this day, breast cancer still causes a health concern for women globally, so we investigated some of the diagnostic variables and inflammatory cytokines in adult females with breast cancer. The study was conducted on 100 adult participants; they were divided into two groups. The first included 60 breast cancer patients and the second 40 healthy women as control, they were attending the designated cancer center at Baquba governmental hospital and Al Shafaa private hospital, Iraq. Blood was collected from both groups, and the following diagnostic parameters were measured CRP, ferritin, IL-6, and TNF-α for the purpose of diagnosis or confirmation of breast cancer. The concluding results demonstrated a remarkable increase in the levels of those diagnostic variables in the serum of cancer patients versus controls individuals at the level of probability $P \leq 0.05$. Thus, we can conclude that these serum parameters may be important indicators of this type of cancer.

Keywords: Breast cancer, inflammatory cytokines, C-reactive protein.

1. Introduction

Carcinoids tumors include are all tumors recognized by abnormal growth and cell proliferation as a consequence of uncontrolled cell divisions, and until today represents a considerable public health concern in the world [1,2]. There is a relationship between cancerous tumors and genetic inheritance, as proven by scientific studies [3]. Breast cancer is an important type of cancer because it represents main cause of cancer mortality among women worldwide [4]. It is the most common non-cutaneous malignancy of breast tissue and manifest in the tubes that carry milk to the teat nipples [5]. This cancer occurs in both genders, but the proportion in females is greater. As for the factors associated with its risk, research has shown a significant decrease in the risk of developing this cancer among the most physically active individuals compared to a higher risk among alcoholics or smokers [6,7]. An unhealthy diet, and high levels of the hormones estrogen, progesterone, and prolactin in the blood, may promote the development of this cancer by stimulating breast cells [8,9]. Epidemiological studies have also shown that contraceptive use and menopause may be linked to the disease [10]. Besides being a life-threatening disease, breast cancer may affect a woman's sense of self-respect and femininity [11]. This type of cancerous tumor is characterized by signs and indicators, including changes in the shape and skin of the breast to become like an orange peel, as well as redness and warmth of the breast skin, and the secretion of a transparent fluid or bloody fluid from the nipple. If the disease spreads in the human body, yellowing of the skin, shortness of breath, pain in the bones and enlarged lymph node are noted [12,13]. Several research papers have proven that high levels of C-reactive protein (CRP) are closely correlating with a higher risk of developing this type of cancer. In addition, CRP assessment is an easy and inexpensive method, so measurement of CRP level is helpful in diagnosing breast cancer [14, 15]. In general, ferritin is the primary protein for intracellular iron storage, and a high level of ferritin has been observed in in cases of inflammatory as well as malignant tumors [16,17]. Notably, inflammatory cytokines have a major aspect that contributes to immune dysregulation-related mortality and leads to many diseases, and the immune system is primarily sensitized to these cytokines, as interleukin-6 is a multifunctional cytokine that aids in inflammatory responses and T-cell differentiation. This interleukin has a critical importance in determining tumor behavior such as growing and proliferation of tumor cells as well as tumor angiogenesis. Its level is high in women with breast cancer [18,19]. Previous studies also indicated that the levels of IL-6 and CRP are higher in breast cancer patients, which are related with a poor prognosis of breast cancer [20]. Tumor necrosis factor-α (TNF-α) is defined as a cytokine secreted by macrophages, natural killer cells, and T cells. This cytokine promotes inflammation, activates the lining of blood vessels and has an important role in carcinogenesis [21]. TNF-α is used as a tumor marker to determine the extent of breast cancer [22]. Through the relationship of this cancer with the variables mentioned above, the current research aimed to study some diagnostic biomarkers in participants (women) with breast cancer in Baqubah, Iraq.

2. Methodology

About a hundred women participated in the current cross-sectional study; sixty were diagnosed with breast cancer versus forty healthy controls, whose ages ranged between (35-70) years. They attended

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the Baquba general government hospital /cancer center and Al Shafaa private hospital in Diyala (Iraq). The period was extended from April 2021 to June 2022, after obtaining the approval of the local health directorate. In addition, informed consent was obtained from each participating woman.

Collection of blood samples

Blood samples were collected from both healthy and patients women by taking approximately 5 cm, and placed in dedicated, anticoagulant-free tubes. Samples were left at 25°C until used, then centrifuged for 10 minutes at 3000 rpm, and after obtaining the necessary sera, they were kept at 20 °C until the diagnostic parameters of MRI, keratin, the level of inflammatory cytokines (interleukin-6 and tumor necrosis factor-alpha) were measured for the purpose of diagnosing breast cancer.

Determination of serum CRP

The CRP-latex assay was used to quantify C-reactive protein (CRP) in the sera of all participants by the interaction that occurred between latex suspension and CRP, as latex clots when it was mixed with sera samples [23].

Determination of ferritin level

The miniVIDAS device was used to measure the concentration of ferritin, and it is one of the most advanced and modern devices that are characterized by accurate results and fast performance, and it works within Enzyme Linked Immunofluorcence ELFA technology, according to the manufacturer bioMerieux.

Estimation of the level of cytokines

The levels of IL-6 and TNF-α were assessed by ELISA technology by the double ELISA Sandwich antibody method. When the results were read, a Microelisa strip of IL-6 antibody was coated, then samples were added to an appropriate Microelisa plate fused with the specific antibodies, then IL6-HRP conjugated reagent and TNF-α were added to each. The T plate was well etched and Microelisa tested and incubated, then it was washed well and then crosogen solution A, B was added, the color of the liquid turned blue. After adding the stop solution, the color turned yellow, and then the absorbance was assessed at a wavelength of 450 nm, in which the value of the intensity of the absorbance was proportional to the concentration of IL-6 and TNF-α in serum.

3. Data Analysis

The data of all examinations were processed statistically by the SPSS (version 24) program, the standard deviation (SD) of the mean was determined, and the differences between both study groups were calculated using an appropriate non-parametric test at the probability level $P \leq 0.05$.

4. Results

Our study recorded the average of the level of CRP was (11.64 ± 1.52) mg /L for patients group, while the control normal group was (6.18 ± 1.42) mg / L, as explained in Table (1).

Table 1: Results of diagnostic variables and inflammatory cytokines for study participants

Parameter	Groups		P-Value
	ControlN=40	PatientsN=60	
CRP (mg/L)	6.18±1.42	11.64±1.52	P ≤ 0.05
Ferritin (ng/ml)	15.66±8.065	39.89±18.715	P ≤ 0.05
IL-6 (Pg/ml)	30.336±8.358	44.852±10.979	P ≤ 0.05
(TNF-α) (Pg/ml)	35.358±11.164	57.739±15.805	p ≤ 0.05

These results showed a significantly higher level of serum CRP of patients group compared to control individuals (Figure 1

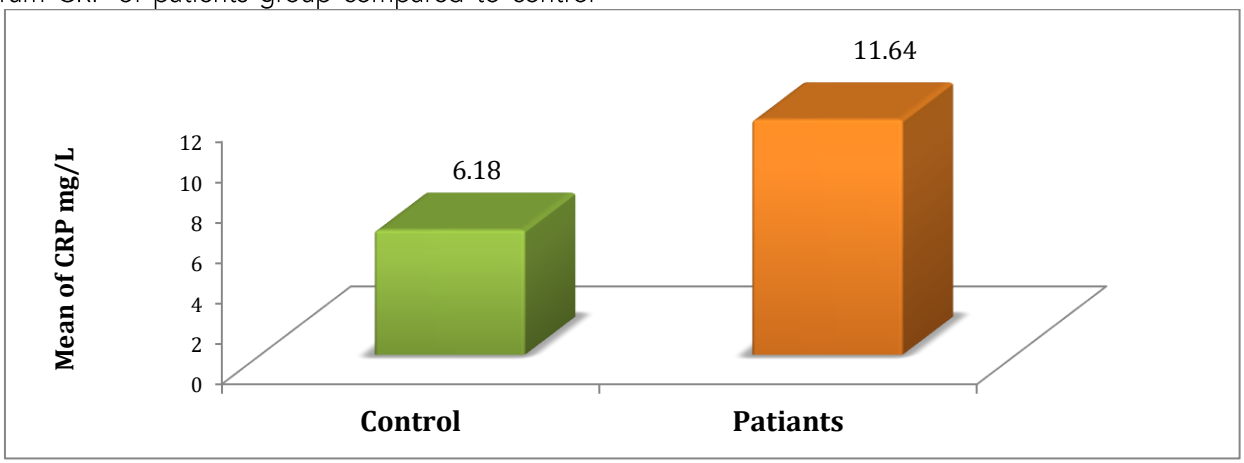

Figure 1: The average level of CRP in the serum of the participants.

The mean of ferritin level was (39.89 ± 18.715) ng/ml among women with breast cancer, while the control women group was (15.66 ± 8.065) ng/ml (Table 1). Thus, there is a clear increase in the ferritin serum level of patients group compared to control group, as shown in Figure (2).

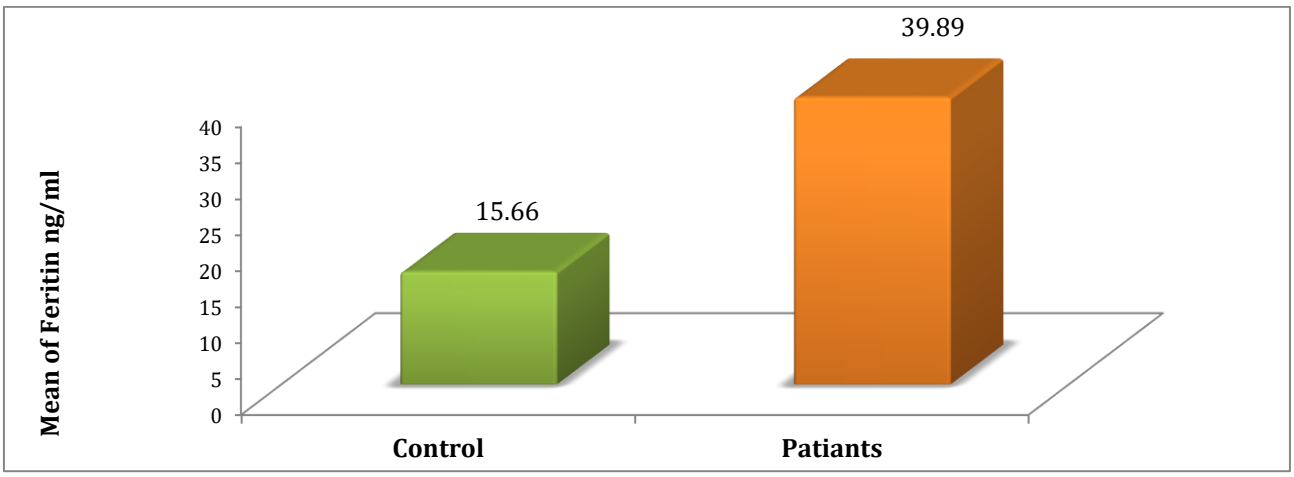

Figure 2: The average level of ferritin in the serum of the participants.

The mean standard deviation of interleukin-6 level was (44.852 ± 10.979) pg/ml among patients with breast cancer, while the control women was (30.339 ± 8.358) pg/ml (Table 1). Therefore, the results showed a considerable raise in level of serological IL-6 of cancer patients compared to healthy individuals, as displays in Figure 3(3).

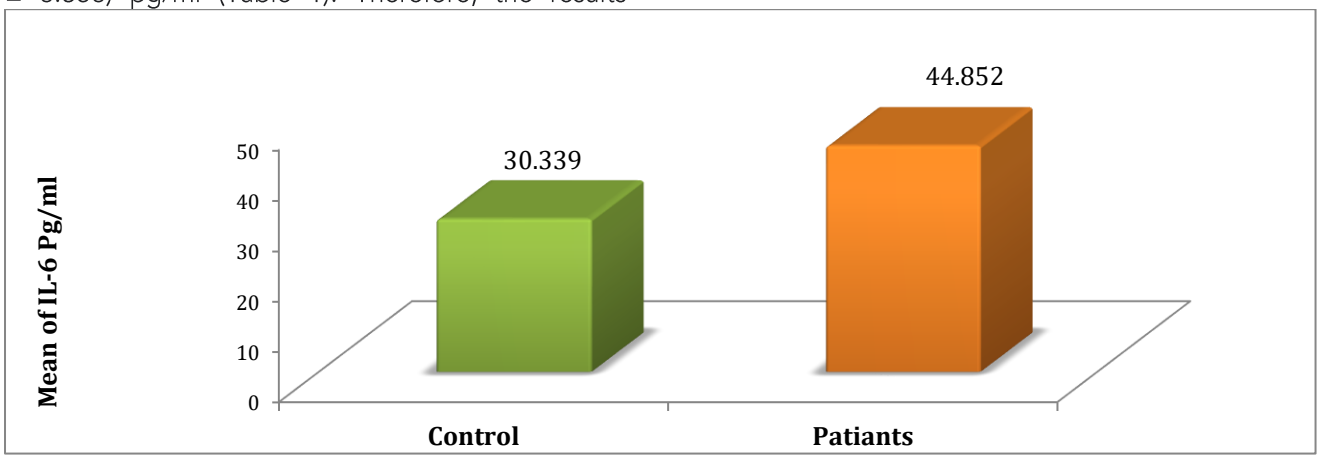

Figure 3: The serological level of IL-6 of the participants.

The mean level of TNF-α was (57.739 ± 15.805) pg/mL for cancer patients group, while the control group was (35,338 ± 11.164) pg/mL (Table 1). Results recorded a remarkable increase in the level of tumor necrosis alpha of patients group versus healthy control group, as in Figure (4).

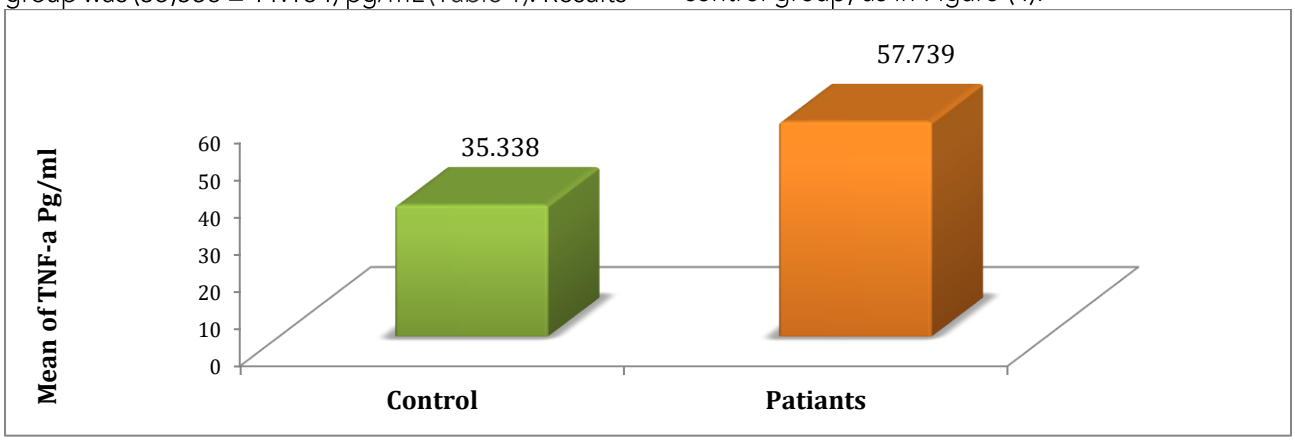

Figure (4): The average levelof TNF-α in the serum of the participants.

5. Discussion

In general, chronic inflammation may be one of the major factors contributing to the progression of carcinomas. Besides, inflammatory passageway have a role in the pathogenesis of breast cancer, and CRP is synthesized after acute inflammatory -reactive protein (ARI) synthesis in hepatocytes as response to cytokines. It is send out from leukocytes into the tumor microenvironment, where CRP concentrations increase in the circulation in response to acute infection, and has prognostic value in breast cancer patients [24,25]. On the other hand, the main reasons that have an effect on the increase in C-reactive protein concentration in patients women compared to healthy women are due to fatigue or exertion [26] where fatigue is one of the harmful effects of cancer treatments and some results indicate its involvement

in inflammatory processes as contributors to cancer-related fatigue [27,28]. The results of our current study are in agreement with those of Mikkelsen et al. [29], Allin et al. [30], and Celik et al. [31], as they confirmed the high serological level of C-reactive protein of patients suffering from breast cancer. As for the evaluation of ferritin, our founding in the current study are in agreement with the results of Fadavi et al [32] and George et al [33], who confirmed in their study an elevated ferritin level in breast cancer patients, as ferritin helped assess and monitor the severity of breast cancer, it is a multifunctional protein with potential roles in proliferation, angiogenesis, and immuno-suppression. Serum ferritin levels are elevated in many cancer patients, especially breast cancer, and high levels are associated with aggressive disease and poor clinical outcomes. Besides, ferritin can be an outstanding target for cancer medication due to its down-regulation can deactivate the tumor-supportive microenvironment, kill cancer cells, and augmentation susceptibility to chemotherapy [34]. IL-6 is an interleukin that has an important role in breast cancer development by inducing apoptosis and stimulating angiogenesis in tumors [35]. The serological level of IL-6 increases of patients with breast cancer, and the tumor develops and spreads, because IL-6 acts as an anti-inflammatory to start the repair process of the affected tissues of the tumor, and this leads to an increase in the serum level of interleukin [36].Our study results were in line with those of Hussain et al [37], and Thwani [38], which showed that IL-6 levels were higher in women with breast cancer than in healthy people. Also, the results of our study were supported by a previous study by Hassan [39], and another by Alkhafaf et al [40], which showed that TNF-α levels were raised in patients with breast cancer versus healthy samples, and anti- TNFs were used as therapeutic agents in solid tumors, but patients who had high TNF-α levels failed to respond to infliximab, due to the body's consumption of circulating anti-TNFs and tumor development [41]. In women with breast cancer, TNF-a blocks the programmed cell death pathway and this leads to the spread of cancer cells to different parts of the body [42], and recent studies have shown that anti-TNF therapies work to manage autoimmune diseases, including cancer, and that inhibiting TNF receptor is a safer and more effective treatment [43]. TNF-α B (NF-kB) is activated when affected cells are exposed to cancer [44], and nuclear factor kappa (NF-KB) have a clear role in inflammation, immune regulation, cell differentiation, and tumor formation [45], and contributes to the proliferation of cancer cells. It also generates neoplastic angiogenesis in cancer cells, stimulating cell proliferation and preventing programmed cell death [56].

6. Conclusions

We concluded through this study that the levels of the following diagnostic serum biomarkers: CRP, ferritin, IL-6, and TNF-alpha were statistically superior in the patients with breast cancer group versus the control normal group. Thus, we can conclude that the levels of these serum variables and cytokines may be important prognostic indicators for breast cancer.

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