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Assessment of Cancer Antigen 15-3 (CA15-3) and Tumor Necrosis Factor-A (TNF-A) as Markers in Women with Breast Cancer

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ABSTRACT

Background: *Breast cancer is still the most common kind of cancer in women, and its incidence is gradually increasing globally. The study aimed to assess cancer antigen 15-3 (CA15-3), and Tumour necrosis factor-α (TNF-α) as markers in women with breast cancer.* ***Materials and methods:*** *This study is case-control design was conducted at National Hospital for Oncology and Hematology in Najaf and AL-Sadder Medical City Hospital. A convenience sampling of 160 samples. The study comprised two groups include 80 patients with breast cancer, 80 healthy controls. Five millilitres of venous blood were collected from every individual in the study consecutively, the blood distributed into: gel tubes (6ml). The blood in tubes is centrifuged for 10 minutes at 3000 rpm. CA 15-3 was measured by utilizing ELFA technology (enzyme-linked fluorescence assay). Tumor necrosis factor-α (TNF-α) were measured by ELISA kit, the biochemical kit used in the study for performed by a company Sun long Biotech Co, Ltd.* ***Results:*** *The results reveal that patients had significantly higher mean CA15-3 levels (30.269±10.666 Ku/l) than controls (16.901±5.742 Ku/l). Regarding TNF-a, the results found that patients had significantly higher mean TNF-a levels (35.510±12.749 Pg/ml) than controls (8.136±3.647 Pg/ml), at statistically significant P. value <0.001.*

Conclusions: *The present study found that TNF-a and CA15-3 levels were higher significantly in women with breast cancer. Furthermore, the results reveal that patients with metastatic BC had had significantly higher mean CA15-3 levels compared to patients with without metastatic BC and controls.*

Keywords: Breast Cancer, Women, Tumor Necrosis Factor-A, Metastasis.

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INTRODUCTION

Breast cancer is still the most common kind of cancer in women, and its incidence is gradually increasing globally. This disease is becoming more common in women, especially in areas where it has previously had a low incidence in BC. The adoption of Western reproductive practices in these nations is responsible for this growth [1]. Breast cancer accounts for 34.27 percent of newly reported cases among Iraqi females up till 2016, making it the most common kind of cancer among women in the country [2]. The World Health Organization (WHO) has shown that prompt diagnosis and necessary care may lower patient mortality. poor treatment choices and poor early detection procedures are the main causes of Iraq's low breast cancer patient survival rates [3].

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Numerous risk factors for BC were found in research done in Iraq, including advanced age, menopause, marital status, and the first menstrual cycle. When comparing separated or bereaved women to married women, a statistically significant association was observed regarding marital status. It is essential to conduct prompt and routine screenings for women, especially when the cancer is discovered early, to determine the best course of treatment and reduce the mortality rate [4]. Tumour markers are proteins produced by malignant cells or other cells in response to a tumour. Some of these markers are associated with specific organ tumours and are used as diagnostic tools for cancer. They can be easily measured in body fluids like plasma or serum [5]. CA15-3, a kind of carbohydrate antigen, is frequently detected in breast cancer patients. It serves more as a prognosis predictor than a diagnostic marker [6]. Tumour necrosis factor-α (TNF-α) is a significant proinflammatory cytokine present in the tumour microenvironment, playing a role in various aspects of breast cancer progression, including tumour cell growth, survival, epithelial-to-mesenchymal transition (EMT), metastasis, and recurrence [7].

MATERIALS AND METHODS

Study design and setting

This study is case-control design was conducted in Najaf province (National Hospital for Oncology and Hematology and AL-Sadder Medical City Hospital). The data was collected during a period from July to November 2023.

The study groups

A convenience sampling of 160 samples. The study comprised two groups include 80 patients with breast cancer, 80 healthy controls. Patients with BC defined by physician as previously diagnosed by specialist. BC disease selected based on confirmation through histopathology findings. Prior to undergoing chemotherapy or radiation, all patients underwent an examination. While healthy controls were identified as not taking any drugs and had no history of chronic or severe diseases.

Inclusion and exclusion criteria

Inclusion criteria:

This study includes individuals aged 18 years or older (age matched ± 2 years between study groups).

Exclusion criteria

Subjects with chronic diseases, pregnancy, autoimmune diseases, and microbial infections, were excluded from the study. In addition, male sex was also excluded.

Methods of sample collection

After interviewing the members of the study groups, their age, and marital status was taken using a questionnaire designed by the researcher. After that, 5 millilitres of venous blood were collected from every individual in the study consecutively, the blood distributed into: gel tubes (6ml). The blood in tubes is centrifuged for 10 minutes at 3000 rpm.

Immunological Assays

Tumor necrosis factor-α (TNF-α) were measured by ELISA kit, the biochemical kit used in the study for performed by a company Sun long Biotech Co, Ltd, (China). The Cancer antigen 15-3 (CA15-3) test is an automated quantitative assay that measures CA 15-3 Detecting antigenic determinants in human serum by utilizing ELFA technology (enzyme-linked fluorescence assay). It is designed to be used on equipment from the VIDAS series (France).

STATISTICAL ANALYSIS

Microsoft Office Excel 2010 and the statistical program for social sciences (IBM-SPSS) version 27 were used to gather, compile, analyse, and display the data. Following the Kolmogorov-Smirnov normality test and the determination of whether the variables were normally or non-normally distributed, the numerical data were presented as mean, standard deviation, and range. To ascertain the

statistical significance of the data, ANOVA analysis was used to compute the least significant difference (L.S.D.) test. A P value of less than 0.05 was deemed significant. The values of the means difference were compared with the L.S.D. values. On the other hand, if the variable is regularly distributed, the independent samples t-test was used to examine the mean difference between any two groups. To investigate the relationship between two or more category variables, the chi-square test was used. P-values of 0.05 or less were used to determine the significance level, while 0.01 or less was used to determine the very significant level.

In table 1, the results found that there were no significant differences between the percentages of breast cancer patients and healthy controls (p = 0.988). Both groups had similar mean ages, with breast cancer patients aged 52.4 ± 12.0 years (range: 33-79) and healthy controls aged 50.8 ± 10.8 years (range: 32-73), with no significant difference (p = 0.357). Regarding marital status, the results found that healthy controls are more likely to be married compared to patients (64.7% vs. 83.5%) respectively, while breast cancer patients are more likely to be widowed (22.5% cases vs. 6.3% controls). Significant marital status differences exist (p =0.023).

RESULTS

Table (1): The distribution of the patients and control subjects according to demographic characteristics

Demographic characteristics		Groups				P.value
		Patients with Breast cancer		H. Control		
		No.	%	No.	%	
Age groups	<40 y	6	7.5	7	8.8	0.988
	40-49 y	31	38.8	31	38.8	
	50-59 y	16	20.0	16	20.0	
	60-69 years	17	21.3	18	22.5	
	≥70 y	10	12.5	8	10.0	
	Mean ± SD (Range)	**53.4±12.0 (33-79)**		**51.8±10.8 (32-73)**		0.357 #
Marital status	Married	51	63.7	66	82.5	**0.023***
	Virgin	8	10.0	6	7.5	
	Divorced	3	3.8	3	3.8	
	Widow	18	22.5	5	6.3	

***Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.**

#Significant difference between two independent means using Students-t-test at 0.05 level.

In table 2, the results reveal that patients had significantly higher mean CA15-3 levels (30.269±10.666 Ku/l) than controls (16.901±5.742 Ku/l), at statistically significant P. value <0.001. Also, the results found that there was no significant difference between stage 0 and healthy controls (P. value >0.05). While stages (I, II, and III) were gradually higher mean CA15-3 levels compared to stage 0 and healthy controls (P. value <0.001). In addition, the results reveal that patients with metastatic BC had significantly higher mean CA15-3 levels compared to patients with without metastatic BC and controls (P. value<0.001).

Table (2): Comparison between levels of the patients with breast cancer and healthy control according to the CA15-3 concentration

CA15-3			
Groups	**Mean± SD**	**SE**	**P. value**
Patients with BC	30.269±10.666	1.192	**<0.001 ***
Healthy Control	16.901±5.742	0.642	
Stage 0	17.913±3.625 A	0.936	**<0.001 #**
Stage I	23.713±3.344 B	0.748	
Stage II	31.696±2.912 C	0.651	
Stage III	41.786±8.952 D	1.790	
Healthy Control	16.901±5.742 A	0.642	
Metastatic BC	37.302±5.549 A	1.274	**<0.001 #**
Non-Metastatic BC	21.227±4.487 B	0.759	
Healthy Control	16.901±5.742 C	0.642	

(*) Significant difference between two independent means using Students-t-test at 0.05 level.

(#) Analysis of variance (ANOVA); using least significant differences as post hoc test (LSD); Different letter denotes to significant differences less than 0.05.

In table 3, the present results found that patients had significantly higher mean TNF-a levels (35.510±12.749 Pg/ml) than controls (8.136±3.647 Pg/ml), at statistically significant P. value <0.001. At the same time, the results found the TNF-α in all stages (0, I, II, and III) were gradually higher mean levels compared to healthy control at significant difference (P. value <0.001). In addition, the results reveal that all patients whether metastatic BC or not had significantly higher mean TNF-a levels compared to controls (P. value<0.001). while there was no significant difference between Metastatic BC and Non-Metastatic BC (P. value >0.05).

Table (3): Comparison between levels of the patients with breast cancer and healthy control according to the Interleukin-TNF-α levels

Tumar Necrosis Factor alpha (TNF- α)			
Groups	**Mean± SD**	**SE**	**P. value**
Patients with BC	35.510±12.749	1.425	**<0.001**
Healthy Control	8.136±3.647	0.408	
Stage 0	39.647±6.818 A	1.761	**<0.001**
Stage I	35.959±13.905 A B	3.109	
Stage II	31.972±11.526 B	2.577	
Stage III	35.500±15.112 AB	3.022	
Healthy Control	8.136±3.647 C	0.408	
Metastatic BC	37.539±11.429 A	1.932	**<0.001**
Non-Metastatic BC	33.932±13.604 A	2.028	
Healthy Control	8.136±3.647 B	0.408	

(*) Significant difference between two independent means using Students-t-test at 0.05 level.

(#) Analysis of variance (ANOVA); using least significant differences as post hoc test (LSD); Different letter denotes to significant differences less than 0.05.

DISCUSSION

The study observed no age or gender difference between patient and control groups (p >0.05). These findings support by [8] and [9] findings that case and control were matched by age and gender. Gender/age matching is necessary for research design to reduce bias. Researchers may eliminate gender/age-related factors by having equal numbers of men and women in case and control groups. More accurate case-control comparisons with gender/age matching decrease bias and increase research validity.

Widows were more likely to have breast cancer in this research. This supports [10] conclusion that widowed patients had a higher breast cancer risk. For several reasons, widows acquire breast cancer more often. Stress and recurrent disruptions following a spouse's death may weaken the immune system and impair health. Widows may also struggle mentally, prompting them to smoke or miss doctor visits. Social support may decrease without a partner, limiting breast cancer detection and treatment. Thus, psychological stress, lifestyle changes, and less support networks may raise widows' breast cancer risk [11].

The results of this study reveal that patients had significantly higher mean CA15-3 levels compared to controls. These results were compatible with the findings of the previous studies [12] [13] [14] which found that serum levels of CA15-3 was elevated in patients with BC compared to healthy controls. Several causes may raise blood CA15-3 levels, a glycoprotein linked to breast cancer. First, it indicates breast cancer cell existence and progression as a tumor burden indicator. Second, the release of antigenic material from malignant breast tissues into the circulation may raise CA15-3 levels, indicating tumor cell turnover and spreading potential. CA15-3 levels may also be raised by tumor microenvironment inflammation and tissue injury [15].

In addition, the results reveal that patients with metastatic BC had significantly higher mean CA15-3 levels compared to patients without metastatic BC and controls (P. value<0.001). these results are consistent with the study findings conducted in Baghdad [16], which reported that increase in CA15-3 levels is associated to advanced stages of breast cancer. According to [17] found that patients in the late stage of the disease had greater levels of positive serum CA15-3 compared to those in the early stage. Other studies found that the levels of CA 15-3 in the serum varied depending on the stage of breast cancer [18]. CA 15-3 levels exhibit a rise in all tumour types, but in the case of breast cancer, they persistently grow as the tumour progresses [19]. Studies have reported that alterations of tumor marker levels are associated with a patient's therapeutic response, in addition to imaging method evaluation [20].

The present results found that patients had significantly higher mean TNF-a levels than controls. while patients within stages (0, I, II and III) were significantly higher mean TNF-a levels compared to healthy controls (P. value <0.001). In addition, the results reveal that all patients whether metastatic BC or not had significantly higher mean TNF-a levels compared to controls (P. value<0.001). these results are consistent with the study findings conducted in Iraq, [21] which found that the levels of blood and saliva samples of TNF (P<0.001) were significantly higher in breast cancer. Also, these results agreed with the previous studies [22] [23] .

The findings of this study are similar to a study by [24], which reported that patients with breast cancer had graduate higher levels of TNF-a according to malignancy grade and occurrence of metastasis

This suggests that several factors lead to elevated TNF-α levels in breast cancer patients. Firstly, TNF-α is crucial for the inflammatory response, which is linked to cancer formation. Chronic inflammation may promote tumor

growth, invasion, and metastasis by increasing TNF-α levels in the tumor microenvironment. TNF-α may aid in angiogenesis, facilitating the growth of new blood vessels and supplying nutrients to the tumor. TNF-α has a function in apoptotic signaling pathways, meaning disrupting them may help cancer cells survive and fight therapy. The higher prevalence of TNF-α in breast cancer patients shows its complicated role in tumor development and dissemination [25] [26] [27].

CONCLUSIONS

The present study found that TNF-a and CA15-3 levels were higher significantly in women with breast cancer. Furthermore, the results reveal that patients with metastatic BC had significantly higher mean CA15-3 levels compared to patients with without metastatic BC and controls.

Ethical Approval

Under the reference number MEC-56, dated September 20 (09/2023), the study was carried out in accordance with a protocol that was approved by a local ethics committee at University of Kufa/ Medicine College/department of medical microbiology and AL-Najaf Health Directorate.

Authors' contributions

Each author took part in the planning and writing of the manuscript as well as coordinating the data preparation. The final draft of the manuscript was read and approved by the author.

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