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Association of neutrophils, monocytes, and lymphocytes with CA15-3 as a predictor of breast cancer in female patients

Raed Madhi[1,*], Hussein Ali Jumaah[2], Hasan Hanoon Hasan[2]

[1]Department of Biology, Collage of Science, University of Misan, 62001 Maysan, Iraq
[2]Al-Hussein Teaching Hospital, Thi-Qar Health Directorate, 64001 Thi-Qar, Iraq

***Corresponding author**
Raed Madhi, PhD
Department of Biology, Collage of Science, University of Misan, Maysan, Iraq
E-mail: raedsaddam@uomisan.edu.iq

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ABEx Bio-Research Center, Dhaka, Bangladesh

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ABSTRACT

Infiltrated leukocytes, such as neutrophils, monocytes, and lymphocytes can be involved in a variety of tumor development. The present study aimed to investigate the linkage of neutrophils, monocytes, and lymphocytes with CA15-3 as a predictor of breast cancer. The study included 116 patients (58 patients before chemotherapy and 58 patients after chemotherapy. CA15-3 antigen levels were measured, and the grading of breast cancer was identified. In addition, neutrophils, monocytes, and lymphocytes percentage and their correlation to CA15-3 antigen were determined. The results observed a significant increase in the CA15-3 antigen levels. Moreover, the study also showed that CA15-3 antigen levels have a great correlation with grades of breast cancer particularly in grade 3 before treatment. The results also found that neutrophils and monocyte percentages were significantly increased in patients before treatment. However, lymphocyte percentage decreased before treatment and greatly increased after treatment. Additionally, the results showed a positive correlation between neutrophil and monocytes percentage with CA15-3 antigen levels before and after treatment. However, the correlation was negative between lymphocyte percentage and CA15-3 antigen levels. The present findings observed that neutrophils and monocytes have a great association with CA15-3 antigen levels, therefore, targeting these cells could protect against breast cancer development. However, targeting these cells can negatively affect the patient's immune response, thus further studies are needed to study the exact mechanism by which neutrophils and monocytes could enhance breast cancer development. Additionally, increased lymphocytes after treatment might be a good strategy to treat breast cancer.

INTRODUCTION

Breast cancer is considered one of the most prevalent cancers and the second most common cause of cancer death in women. It is indeed one type of cancer that is initiated and developed in breast tissue. Histopathological findings have shown that breast cancer may begin in either the left or right breast [1-3]. Age and breast cancer incidence are strongly correlated, with incidence rising in the age range above 45 years [1, 4]. There are various methods for identifying and tracking the incidence and development of breast cancer. For example, blood tumor indicators like CA15-3 antigen or genetic profiles can be used to diagnose breast cancer. CA15-3 has indeed been shown to be assured for detecting breast cancer [5-7].

There is mounting evidence that the immune system and its constituent parts play an essential role in the initiation and growth of malignant tumors. Cancer indeed triggers an immune response in its microenvironment, but these responses are typically inefficient at eliminating cancerous cells [8, 9]. In both bone marrow and peripheral blood, neutrophils are known to be the most prevalent innate immune cells [10, 11]. They have considerable flexibility and a potent effector response and are instantaneously infiltrated into sterile or infected inflammatory sites. Neutrophils have

a brief lifespan [12], possibly to head off unneeded tissue injury. Therefore, the quantity of neutrophils depends on ongoing granulopoiesis in the bone marrow replenishment. Numerous ways explain how neutrophils are engaged in pathogenesis. In the beginning, neutrophils attracted to the inflamed site produce proteolytic enzymes and generate a lot of reactive oxygen species (ROS), which damages the tissue and makes it more vulnerable to pathogens and can even lead to the emergence of chronic inflammation [13-15]. Numerous cases of severe coronavirus disease 2019 (COVID-19) cases as well as other viral and pulmonary disorders have shown this pathogenic consequence [15, 16]. Neutrophils are frequently found in the tumor microenvironment, but little is known about their function in the development of cancer, and they have typically been viewed as bystanders [17, 18]. Neutrophils are an essential part of developing cancer by causing inflammation and damaging tissues. Neutrophils therefore establish a connection between inflammation and malignancy [19, 20]. Moreover, it has been demonstrated that monocytes and lymphocytes play a key role in systemic inflammatory response [21, 22]. These cells indeed vary in their function with respect to cancer development and metastasis. For instance, lymphocytes have been shown to work as tumor suppressors. In contrast, monocytes can positively participate in tumor cell invasion and metastasis by constructing the microenvironment of the tumor [23, 24]. Thus, the current study aimed to estimate the linkage of neutrophils, monocytes, and lymphocytes with CA15-3 in breast cancer.

MATERIALS AND METHODS

Study design

The current study was carried out in Maysan City, Iraq. Samples were collected from 116 patients, including 58 patients before getting the chemotherapy treatment and 58 patients after getting the chemotherapy treatment who suffered from breast cancer and attended the oncology center in Al-Sader Hospital, Maysan, Iraq. Specialist pathologist staff were diagnosed with breast cancer and based on different approaches; clinical examinations, estimation of CA15-3 levels, and biopsy of the breast. In addition, 40 blood samples from healthy women were also collected as a control.

Ethical approval

The ethics committee of Trigis Medical Center affiliated Maysan Health Department has approved the protocol of the present study (approval number: 2021-02).

Measurement of CA15-3

A commercial kit (EIA-3941, DRG, Germany) was used to measure CA15-3 levels of patients and healthy groups according to the manufacturer's instructions. Briefly, 10 µL of standard, samples, and controls were dispensed into corresponding wells. The amount of assay buffer used for each well was 250 µL. The wells were mixed smoothly and incubated at room temperature for 60min. Then, each well was rinsed with a 400 µL wash solution three times. After washing, each well was incubated with 100 µL of enzyme conjugate at room temperature for 60 min. Thereafter, each well was rinsed with 400 µL wash solution three times and incubated with 100 µL of solution of substrate at room temperature for 30 minutes. Finally, 100 µL the reaction was stopped in each well by adding a stop solution (100 µL). The reaction was read at wavelength 450 nm after ten min of stopping the reaction.

Histological examinations

A biopsy of the breast was collected to confirm the diagnosis of breast cancer in suspected women. Normal breast tissues were obtained from the oncology center/Al-Sader hospital/Maysan and used for comparison by specialist pathologist staff. Breast tissue samples were subjected to fixation in 4% formaldehyde and then dehydration and embedding with paraffin. After, hematoxylin and eosin (H&E) stains were used for staining the tissue samples. Examinations of tissue samples were done under light microscopy. Breast cancer grading was performed according to a standard protocol [25].

Measurement of leukocytes in blood samples

To count and determine leukocytes in blood samples of control and patients, a CELL-DYN (Abbott, Abbott Park, Illinois, U.S.A.) was used by using Multi-Angle Polarized Scatter Separation (MAPSS) technology. Briefly, the identification and differentiation of neutrophils, monocytes, and lymphocytes were performed by using four light scatter detectors. Cell passes through a tiny opening that separates the two electrodes while whole blood is transferred between them. The cell count and volume were performed quickly by measuring the changes in electrical conductance when cells floating in a conductive fluid passed through a tiny opening, which is proportional to the cell volume, and the final results were presented as percentages.

Statistical analysis

SigmaStat 3.5 program was used to perform statistical analysis. A T-test was used to compare two different means. In addition, the LDS method was also used for further comparison. Data are introduced as mean values ± standard error of the mean. $P < 0.05$ is considered as a statistical significance between the groups.

RESULTS

CA15-3 antigen levels in breast cancer patients

It is believed that the CA15-3 is used frequently as a diagnostic biomarker for breast cancer [26]. Serum CA15-3 was measured in breast cancer patients and control groups (Figure 1). Our results demonstrated that serum CA15-3 antigen levels were significantly increased by 3.7-fold and 1.5-fold in patients with breast cancer before getting chemotherapy and patients with breast cancer after getting chemotherapy, respectively (Figure 1).

Breast cancer grading and CA15-3 antigen levels

Histological examinations were performed for further confirmation of breast cancer diagnosis in suspected women with breast cancer (Figure 2). The histological examinations observed that the cells and tissue architecture of grade 1 breast cancer are more similar to normal cells (Figure 2A). However, in grade 2, histological examinations showed that cells and tissue architecture look quite different from normal cells (Figure 2B). Moreover, grade 3 showed a very different look in the cells and tissue architecture in breast tissue (Figure 2C). It was interesting to evaluate the linkage between CA15-3 antigen levels and the grading of breast cancer. CA15-3 antigen levels

were divided into three groups (10-20, 21-29, and ≤30, respectively) (Figures 2D and E). Our results revealed that the levels of CA15-3 antigen were significantly increased ($P<0.05$) in grade 3 as compared to grade 1 in patients with breast cancer before getting chemotherapy (Figures 2D and E). However, the statistical analysis showed no significant differences ($P = 0.461$) among the grading of breast cancer in CA15-3 antigen levels after treatment (Figure 2E).

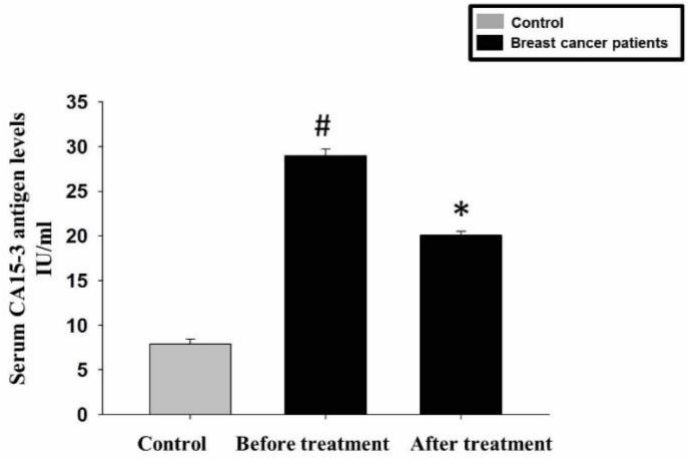

Figure 1. CA15-3 antigen levels. ELISA method was used to measure the levels of CA15-3 antigen in control and patients with breast cancer before and after getting the chemotherapy. Patients with breast cancer are introduced by the black box. Control groups are introduced by the grey box. Data introduced as means ± SEM. #P <0.05 versus control.

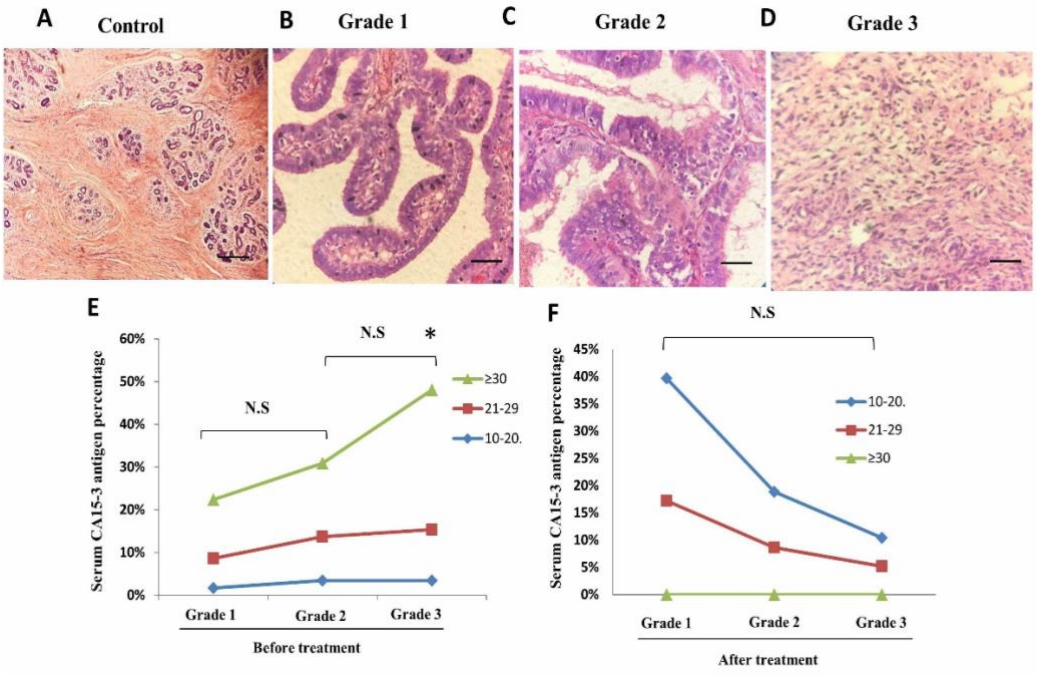

Figure 2. Breast cancer grading and CA15-3 antigen correlation. Sectioning of (H&E) of breast tissue. Scale bar = 50 μm. A) Normal breast tissue, B) Grade 1, C) Grade 2, and D) Grade 3. CA15-3 antigen association into breast cancer grading, E) before getting chemotherapy and F) after getting chemotherapy. Data represented as mean of percentage; the LDS method was used to compare between groups. *P-value <0.05 is considered as a significant difference between the groups.

Estimation of neutrophils, monocytes, and lymphocytes in breast cancer

Neutrophils play a great role in cancer cell development and metastasis. In this study, we examined the percentage of neutrophils before and after treatment in patients with breast cancer (Figure 3A). Our results found that levels of neutrophils were significantly increased ($P<0.05$) in patients with breast cancer before getting the treatment as compared with control (Figure 3A). Moreover, the present results show that the levels of monocytes were substantially increased ($P<0.01$) in breast cancer patients before chemotherapy compared to the control group (Figure 3B). However, the results also found that the levels of lymphocytes were increased by 1.5-fold as compared to the control group after treatment (Figure 3C).

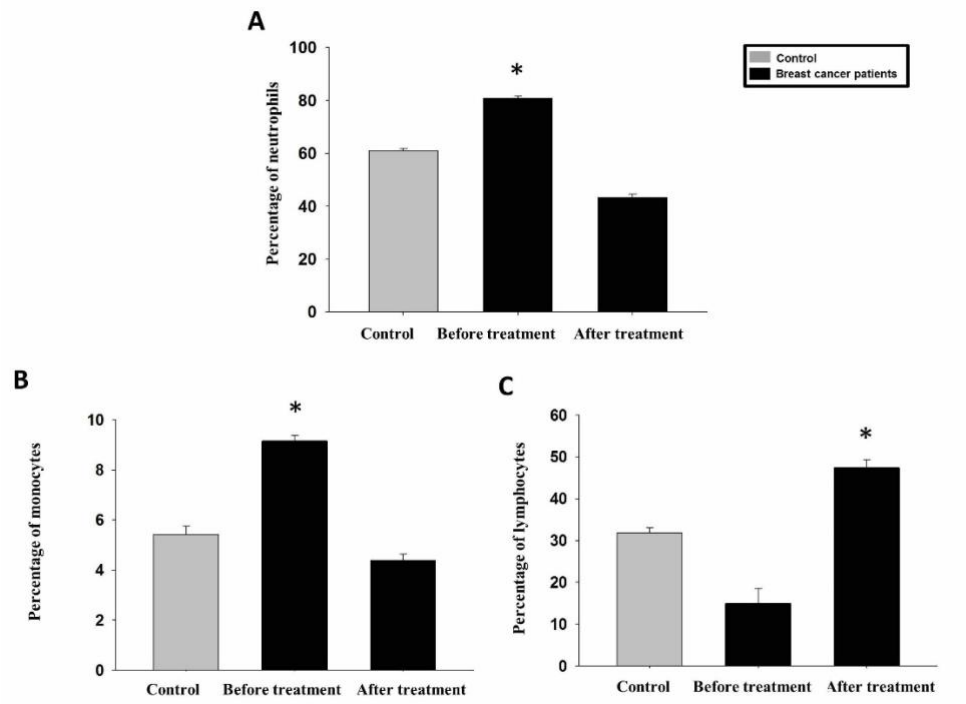

Figure 3. Estimation of neutrophils, monocytes, and lymphocytes in the blood. Measurement percentage of A) neutrophils, B) monocytes, and C) lymphocytes in both control and patient groups. Control group (gray box). Patients with breast cancer before and after getting the chemotherapy (black box). Data represent means ± SEM. *A p-value of <0.05 is considered a significant difference between the groups.

Correlation of neutrophil, monocytes, and lymphocytes with CA15-3 antigen levels

It was important to investigate the relationship among neutrophils, monocytes, and lymphocytes with serum CA15-3 antigen levels in breast cancer patients. Linear regression was used to analyze the relationship of neutrophils, monocytes, and lymphocytes with serum levels of CA15-3 antigen (Figure 4). Interestingly, the statistical analysis showed a strong correlation ($y = 0.981x + 52.352$ $R^2 = 0.7204$ and $y = 3.2024x - 22.109$ $R^2 = 0.7512$) between neutrophils and CA15-3 antigen levels before and after getting the chemotherapy, respectively (Figure 4A and B). In addition, our results revealed that there is a significant correlation ($y = 0.2342x + 2.3657$ $R^2 = 0.5628$ and $y = -0.6086x + 16.817$ $R^2 = 0.7486$) between monocytes and CA15-3 antigen levels before and after getting the chemotherapy, respectively (Figure 4C and D). In contrast, the statistical analysis found a negative correlation ($y = 0.1195x + 11.458$ $R^2 = 0.0502$ and $y = -0.0776x + 48.993$ $R^2 = 0.0011$) between lymphocytes and CA15-3 antigen levels before and after getting the chemotherapy, respectively (Figure 4E and F).

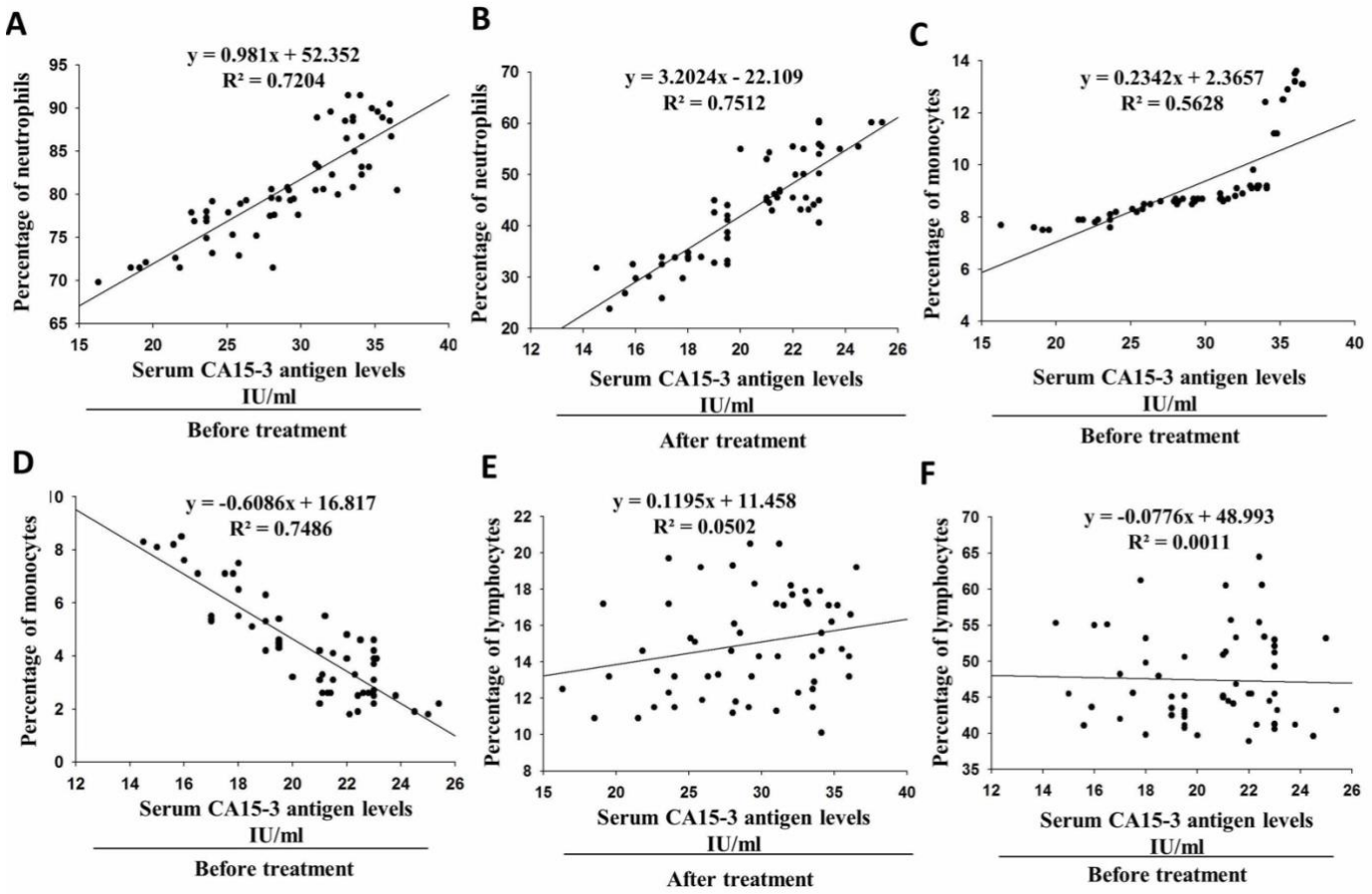

Figure 4. Linkage of neutrophils, monocytes, and lymphocytes with CA15-3 antigen levels. Correlation of CA15-3 antigen levels into neutrophil A) before getting chemotherapy (y = 0.981x + 52.352, R^2 0.7204) and B) after getting chemotherapy (y = 3.2024x - 22.109, R^2 = 0.7512), monocytes C) before getting chemotherapy (y = 0.2342x + 2.3657, R^2 = 0.5628) and D) after getting chemotherapy (y = -0.6086x + 16.817, R^2 = 0.7486), and lymphocytes E) before getting chemotherapy (y = 0.1195x + 11.458, R^2 = 0.0502) and F) after getting chemotherapy (y = -0.0776x + 48.993, R^2 = 0.0011). Linear Regression was used to compare between groups.

DISCUSSION

In the current study, the data demonstrated that CA15-3 levels have an association with the diagnosis of breast cancer. Furthermore, neutrophils and monocytes were greatly increased in the blood of patients with breast cancer before treatment and have an association with levels of serum CA15-3 antigen. Additionally, lymphocytes were increased after treatment and have a negative correlation with levels of serum CA15-3 antigen. Therefore, an increased percentage of neutrophils and monocytes could be used as an indicator for breast cancer diagnosis and increasing lymphocyte levels after chemotherapy might provide a successful strategy to treat patients with breast cancer.

Moreover, breast cancer cells have been found to express high levels of CA15-3 antigen compared to the normal [26]. In the current study, our results revealed that the CA15-3 levels significantly increased in breast cancer patients. In addition, CA15-3 antigen levels showed an increase in different grades of breast cancer, however, the increase of CA15-3 antigen levels was significant particularly in grade 3 before chemotherapy treatment. This could explain why the CA15-3 be utilized as a biomarker for the diagnosis of breast cancer.

It is well known that the most abundant leukocytes in the blood are represented by neutrophils, which are regarded as the first line of defense when the body is exposed to

infection and inflammation [27-29]. An inflammatory response caused by invasive bacteria draws neutrophils from the bloodstream into the tissues. The pathogen is then eliminated by neutrophils using a variety of methods, chiefly phagocytosis, the release of microbicidal agents, and the development of neutrophil extracellular traps (NETs) [13, 14, 30]. Additionally, proteinases are released by activated neutrophils into the tissue around them, harming the host [31, 32]. Moreover, neutrophils and monocytes were shown to play a significant role in regulating cancer cell development [33]. In the present study, it was important to estimate the percentage of neutrophils and monocytes in breast cancer patients and their correlation to the levels of CA15-3 antigen. Interestingly, the current results demonstrated that neutrophils and monocytes were significantly increased in the blood of patients with breast cancer before getting the treatment. Moreover, we investigated the association between the percentage of neutrophils and monocytes with CA15-3 levels. Our findings showed a great correlation between the percentage of neutrophils and monocytes CA15-3 antigen levels. Notably, neutrophils and monocytes have been reported to be infiltrated into different kinds of cancers. It was difficult to envision those neutrophils, because of their short-lived, could have an impact on chronic and progressive diseases like cancer in the early investigations. However, it has lately become evident that tumor-related neutrophils play important roles in malignant illness. This newfound interest is partly due to the realization that cancer-associated inflammation is a crucial component for the growth of many tumors [34, 35] and is a distinguishing feature of cancer. In addition, we have also examined the percentage of lymphocytes in patients with breast cancer. Our results found that the percentage of lymphocytes greatly increased after treatment. It is thought that chemotherapy destroys the immune cells, particularly innate immune cells that represent the first defense lines. However, cell debris that results from the cytotoxic effects of chemotherapy releases signals that can lead to the actuation of dendritic cells (DCs) to engulf the dying cells. Subsequently, DC, as antigen-presenting cells, stimulate lymphocyte recruitment such as helper T cells.

CONCLUSIONS

In conclusion, the current findings demonstrate a great association between CA15-3 levels and the grading of breast cancer. Moreover, neutrophils were highly elevated in breast cancer, and therefore, an increased percentage of neutrophils besides the levels of CA15-3 antigen might be a good strategy to be used as an indicator for breast cancer diagnosis. However, the limitation presented in the current study is that the conclusion was based on one diagnostic marker. Thus, further studies are needed to study the role of neutrophils in breast cancer development based on different diagnostic markers. In addition, according to the current findings, neutrophils might be involved in the development and spread of breast cancer. Thus, the present results emphasize the role of the immune system in the development of cancer and the possibility of immunotherapies to treat breast cancer, even though the exact mechanisms are still being investigated.

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AUTHORS CONTRIBUTIONS

RM, HAJ, and HHH assisted in conducting the experiments, performed the statistical analysis and data visualization, and wrote the manuscript. RM designed and conducted all of the experiments and wrote the manuscript. All authors have read and approved of the final manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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