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Tenascin-C As A New Marker For The Diagnosis And Treatment Monitoring Of Breast Cancer

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Abstract

All over the world, the most frequent cancer and death cause among women is breast cancer. One of the most effective ways to avoid breast cancer is to diagnose it early. Tumor markers are the simplest and easiest methods for the clinical diagnosis of breast cancer, however, the currently used markers lack accurate specificity and sensitivity and their results are still controversial. Accordingly, the present study aims to investigate the possibility of using tenascin-c (TNC) protein as a new marker for the diagnosis in treatment monitoring of breast cancer. In this study, blood samples were collected from thirty age-matched untreated breast cancer patients, thirty hormonally treated breast cancer patients and thirty apparently healthy female subjects to serve as a control group. Serum levels of TNC, cancer antigen 15-3 (CA15-3), human epidermal growth factor 2 (HER2) and estradiol (E2) in all of the study subjects using enzyme linked immunosorbent assay (ELISA). Serum levels of TNC, CA15-3, HER2 and E2 were significantly increased in untreated breast cancer patients compared to the control group, however, their levels were significantly decreased in the hormonally treated breast cancer patients compared to the untreated patients. Tenascin-c may be used as a new marker for the diagnosis and treatment monitoring of breast cancer.

Keywords: tenascin-c serum levels, breast cancer, tumor marker.

Introduction

Breast cancer is the most common cancer among females worldwide. Breast cancer is the most common cancer with more than 2.2 million cases in 2020 [1]. There are many causes of cancer such as mineral concentration[2]. Cancer is a major public health issue that affects people all over the world[3]. By 2030, the worldwide cancer burden is expected to increase to 21.7 million new cases and 13 million deaths[4]. All over the world, the most frequent cancer and death cause among women is breast cancer, it accounts for 23% of total cancer cases and 14% of cancer deaths [5]. In Iraq, breast cancer ranks the first malignant cancer

that affects women which equals to 34.3% of female cancers [6]. Breast cancer is the leading cause of cancer mortality, resulting in more than 14% death annually in Iraq [7].An early diagnosis is one of the most best ways to manage breast cancer. Breast cancer is a multi-stage, multi-type cell process, and it is still hard to stop it all over the world. In some advanced nations, breast cancer patients with early detection and treatment have a relative survival rate of 5 years exceeding 80%. Major breast cancer progress and the advancement of preventative measures over the last decade has been achieved [8].

A tumor marker is a biochemical indicator detected in the blood, body tissues, urine which is raised when one or more kinds of cancer are present. It can be generated by the tumor or by the host in reaction to the tumor [9].To detect tiny cancers and aid in early diagnosis or screening, Precise and sensitive must be the perfect tumor marker. There are only a few biomarkers that are distinct to a single tumor.Tumors of the similar kind of tissue are the main part of the markers. They can be seen more in cancer tissue and blood concentrations than in healthy people's blood in cancer patients. Cancer indicators are most effective for determining how far the disease has progressed after first chemotherapy and radiotherapy, as well as monitoring subsequent treatment methods [10].Early diagnosis of primary and recurrent breast cancer is critical in clinical practice, as it can be utilized to inform treatment decisions while tumor burden is low and patients are most prone to respond to adjuvant therapy [11]. Cancer indicators are inexpensive way of information that can be used to track the progression of a disease, determine prognosis, and aid in the planning of treatment. For effective utilization and appropriate interpretation of results, it is necessary to understand the individual test characteristics and limits [12]. The true usefulness of tumor markers in breast therapeutic interventions has been doubted because of the low diagnostic sensitivity for early disease[13].

CA 15-3 is a predictor of recurrence and advanced breast cancer that is independent of other factors [14]. This CA15-3 marker is employed in the surveillance of patients with diagnosed breast cancer and treatment monitoring, and it is regularly used for assessing treatment responses and postoperative disease recurrence during patient follow-up. In around 70% of asymptomatic patients, detecting a rise in the level of CA15-3 can be utilized to diagnose metastatic illness [15].

Tenascin-C (TNC) is a glycoprotein found in the extracellular matrix (ECM) that is involved in cell proliferation, migration, and tumor invasion in a variety of malignancies. TNC is one of the most overexpressed proteins in breast cancer, implying that this ECM molecule

has a function in cancer pathogenesis [16].The extracellular matrix (ECM), which provides essential regulatory cues for cellular responses, is becoming identified as a crucial role in cancer growth and metastasis. Signaling pathways' functional outcomes are depend very much on context and can be influenced by ECM composition. TNC is an ECM glycoprotein with a complex relationship to cancer that has been known since its discovered in the eighties [17].

TNC is a pleiotropic molecule that has been linked to a variety of biological functions, including the promotion of metastasis. TNC function is diverse, including functions such as adhesion and migratory pathways regulation, formation of new blood vessels, and immune response regulation. These biological processes may be required at different times during the metastatic cancer cells' life cycle [18].At the major tumor site, anti-adhesion TNC features contribute to intracellular alteration in cancer cells that promote the synthesis, enhance cell migration and invasion activity of actin-rich filopodia. TNC is also associated with an increase in the proliferation of cancer cells and an increase in tumor angiogenesis. Autocrine TNC improves the stability of cancer cells in a medium that can exert selection pressure at the secondary organ site. TNC triggers signals from stem/progenitor such as Notch and Wnt in breast cancer, which promote growth of micrometastases[19].

Being an important extracellular matrix protein that plays multiple and different roles in the pathogenesis of cancers, tenascin-c was measured in sera of breast cancer patients aiming to investigate the usefulness of using tenascin-c as an indicator of breast cancerdetection and treatment monitoring.

Methods

Study subjects

This study included 90female subjects divided into three groups; the first group included 30 patients with newly diagnosed breast cancer (before treatment) with a mean age 54.93±5.76 years and an age range of 46 – 68 years. The second group included 30 patients with breast cancer treated with trastuzumab. The mean age of the second group was 52.20±5.38 years and an age range of 45 – 65 years. The third group included 30 apparently healthy female subjects to serve as the control group. The mean age of the control group was 52.67±5.76 years with an age range of 45 – 69 years. The subjects enrolled in this study were attending Al-Anbar Teaching Hospital / Tumor Center. A verbal consent was taken from each study subject enrolled in this study.

This study was approved by the Ethics Committee in the Applied Science Department / University of Technology and Ministry of Health, Baghdad, Iraq.

Exclusion Criteria:

The following subjects were excluded in order to avoid any possible effect on the results of the study: surgically treated cancer patients, patients with breast cancer after radio and chemotherapy, patients with metastatic breast cancer, patients with other types of cancer, patients with benign breast tumors and patients under hormone replacement therapy.

Collection and storage of blood samples:

Five milliliters of venous blood were taken from each patient and healthy control by vein puncturing using plastic disposable syringes. The blood was transferred into a gel tube and it was left for (15 – 30) minutes at room temperature in order for the clotting process to start. After that, the sample was centrifuged at 4000 ×g for (15) minutes to separate the serum. The sera collected were divided into aliquots and stored at (-20 ºC) until assayed.

Measurement of TNC, HER2 and E2.

Tenascin-c serum levels were measured using Human Tenascin C ELISA Kit (RayBiotech) according to the manufacturer's instructions.

CA15-3, HER2 and E2 serum levels were measured using Human Mammary Carcinoma Marker-CA153,Human Epidermal Growth Factor Receptor 2 (Her2), Human Estradiol (E2) ELISA Kits respectively (Melsin Medical Co.) Under the protocol of the kits producer.

Statistics:

Biochemical data were analyzed by SPSS (statistical package for social sciences) software, version (25). One-Way ANOVA was used in order to assess the results of this study.

Results and discussion

Serum levels Tenascin-c, CA15-3, HER2 and E2 measured in patients and controls are shown in table 1.

Table 1: Serum levels Tenascin-c, CA15-3, HER2 and E2 measured in patients and controls

Parameter	Group (A) Mean ± SD N=30	Group (B) Mean ± SD N=30	Group (C) Mean ± SD N=30	p-value (A vs B)	p-value (A vs C)	p-value (B vs C)
TNC (pg/mL)	97.93±36.64	314.37±201.02	204.97±52.59	0.000*	0.001*	0.001*
CA15-3	17.62±5.33	40.81±4.89	32.47±2.26	0.000*	0.000*	0.000*

(U/mL)						
HER2 (ng/mL)	0.43±0.10	0.63±0.11	0.36±0.10	0.000*	0.019*	0.000*
E2 (pg/mL)	14.91±5.26	38.62±9.16	27.42±6.96	0.000*	0.000*	0.000*

***Significant at (p ≤ 0.05). Group (A): Controls, Group (B): Breast cancer patients before treatment, Group (C): Breast cancer patients after treatment, SD: standard deviation, N: number of subjects.**

As it can be seen from table (1), there was a significant ($p < 0.000$) increase in the serum levels of TNC in untreated breast cancer patients (314.37±201.02 pg/mL) compared to the control group (97.93±36.64 pg/mL). There was a significant decrease ($p = 0.001$) in the serum levels of TNC in treated patients group (204.97±52.59 pg/mL) compare to the untreated patients group (314.37±201.02 pg/mL). However, the serum levels of TNC were still significantly higher ($p = 0.001$) in the treated patients group compared to the control group.

There was a significant ($p < 0.000$) increase in the CA15-3 blood concentration in untreated subjects having breast cancer (40.81±4.89 U/mL) compared to the control group (17.62±5.33U/mL). there was a significant decrease ($p < 0.000$) in the serum levels of CA15-3 in treated patients group (32.47±2.26 U/mL) compare to the untreated patients group (40.81±4.89U/mL). However, the serum levels of CA15-3 were still significantly higher ($p < 0.000$) in the treated patients group compared to the control group.

There was a significant ($p < 0.000$) increase in the serum levels of HER2 in untreated breast cancer patients (0.63±0.11 ng/mL) compared to the control group (0.43±0.10 ng/mL). there was a significant decrease ($p < 0.000$) in the serum levels of HER2 in treated patients group (0.36±0.10 ng/mL) compare to the untreated patients group (0.63±0.11 ng/mL). The serum levels of HER2 were significantly decreased ($p < 0.019$) in the treated patients group compared to the control group.

There was a significant ($p < 0.000$) increase in the serum levels of E2 in untreated breast cancer patients (38.62±9.16pg/mL) compared to the control group (14.91±5.26pg/mL). There was a significant decrease ($p < 0.000$) in the serum levels of E2 in treated patients group (27.42±6.96pg/mL) compare to the untreated patients group (38.62±9.16 pg/mL). However, the serum levels of E2 were still significantly higher ($p < 0.000$) in the treated patients group compared to the control group.

Even though the CA15-3 blood concentration were elevated in subjects having breast cancer compared to the control group, The function of the serum marker is still debated in the surveillance of breast cancer. It is omitted from key international directives such as ASCO, NCCN and ESMO from routine use as as a follow-up strategy for asymptomatic women after breast cancer therapy [20-22]. Prognosis and illness monitoring while treatment are the most important uses.Although there are not enough studies to show a link between the early diagnosis of relapses and improved outcomes, many doctors still count on serial assessment of tumor markers as a simple add-on test that can be expected to diagnose a recurrence with up to 9 months of lead time.CA 15-3 was the most commonly utilized and suggested of the serum tumor markers for breast cancer. In a recent meta-analyse, Li conducted 36 studies in 12,993 participants, high CA 15-3 was demonstrated to be linked to poor DFS and overall survival (OS). Although some writers support the frequent use of tumeor markers, the degree of this advice is uncertain and doctors have no standards for integrating tumor marker assessment in the monitoring of breast cancer[23].

In roughly 70% of asymptomatic individuals the identification of an increase in level CA15-3 can be used to detect metastatic illness. In several other cancers, including ovarian cancer, increased levels CA 15-3 have also been observed in addition to breast cancer[24-27].

In individuals suffering from several forms of advanced adenocarcinoma, such as ovarian, pancreatic, gastric, or lung cancer, increased concentrations of CA15-3 may be found[28,29]. Since the levels of CA15-3 only rise in 10% of people with stage 1 cancer, rapid recognition is of little utility [30].

Expression of TNC is temporary and limited to the modelingduration phase in tissue regeneration and repair processes. For example, TNC levels are significantly decreased once wound healing is complete, and TNC is almost non-existent in scar tissue. Tenascin-c, on the other hand, may not be regulated in the same way in cancer, resulting in long-term TNC activity. TNC has been demonstrated to alter a variety of aspects of cancer cell biology, as well as alter many pathways required for cancer cells to spread to distant tissues and create overt metastasis [31].

From this prospective, we estimated the levels of TNC in the blood of breast cancer patients to assess the clinical significance of using TNC as an indicator for the identification and prognoses of breast cancer and according to our results, TNC can be used as a diagnostic and prognostic marker for breast cancer. This study presents the first utility of TNC serum

levels as a marker for breast cancer and to date there has been no previous studies to measure the levels of TNC in the blood of breast cancer patients and there are no studies to confirm or contradict our results.

Estrogens, both endogenous and exogenous, have been linked to an increased risk of breast cancer.Estradiol (E2) is the most physiologically active stimulator in estrogen-positive breast cancer. E2 has the most physiological action between estrogenous substances.The interaction between E2 and ERs causes ER to be linked with specific DNA sequences in the target gene promoter where it works as a factor of transcription. It depends on the organ whether transcription is controlled up or down. E2 is supposed to help the development of positive ER breast cancer [32].

The previous observational and experimental investigation supports the relationship of elevated serum steroid hormones, particularly for estrogen receptor (ER) positively tumors, to increased risk of post-menopausal breast cancer[33].

In human breast cancer, the proto-oncogene HER-2 is the most typically amplified oncogene. HER-2 is a transmembrane protein that belongs to the family of epidermal growth factor receptors. In 15–30% of invasive breast tumors, the HER-2 gene is either amplified or overexpressed. All patients with breast cancer must have their HER-2 status determined. The guidelines of ASCO to determine HER 2 status included the immunohistochemistry which assesses the over-expression of full-length oncoprotein (p185) from HER-2 and fluorescence in situ (FISH) which measures the number of HER-2/neugene copies [34].All patients must have their HER-2 tumor expression determined in order to be considered for treatment with Herceptin® (trastuzumab). Herceptin® is a humanized monoclonal antibody, which binds with a close connection to the HER-2 extracellary domain and hampers its signaling function. Herceptin® is presently utilized in late breast cancer therapy of HER-2-positive tumors and in adjuvant therapy of HER-2 positive patients of early breast cancer[35]. This explains the decrease in HER2 serum levels in the patients group treated with trastuzumab in this study.

Conclusions

According to the results of the present study, Tenascin-c may be used as a marker for the diagnosis and treatment monitoring of breast cancer.

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Conflict of interests

The authors declare that they have no conflict of interests.

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