

Immunological study of CA-15.3 and CEA tumor markers among post-operative breast cancer patients

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Abstract : The burden of breast cancer is increasing in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women; comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality. This case-control study was arranged to investigate the possible role of selected genetic and immunological parameters in a random samples of patients with breast cancer in the Al-Diwanyia province. Five ml blood samples obtained from fifty females with breast cancer in post-operative stage attending the outpatient department of oncology in Al-Diwaniya teaching hospital have been recruited in the study and compared to 50 healthy control females, ages of patients and control were ranged between 18-80 years. 6ml blood samples were assessed for serum measurement CA-15.3 and CEA tumor markers by using Fully-auto chemiluminescence immunoassay – CMIA. In the association between breast cancer and selected immunological markers our results showed a significant association between serum concentration of tumor markers (CA-15.3 and CEA) and breast cancer disease ($p < 0.05$) in comparison with control group. The mean serum CA-15.3 and CEA were significantly higher in patients as compared to both control groups P value < 0.0001 . The statistical results show no significant association between CA 15-3 and CEA in patients group ($p = 0.185$), but moderate positive correlations is appeared ($r = 0.2432$). In the other hand the association between patients age and selected immunological markers study in our study (CA-15.3 and CEA) and the

results showed to the serum levels of CA-15.3 and CEA not effected strongly by Age ($r= 0.20$ and $r= 0.114$ respectively).

الخلاصة: إن ثقل مرض سرطان الثدي يزداد في جميع دول العالم حيث يعتبر هو أكثر مرض تتعرض إليه الإناث من بين بقية أنواع السرطانات حيث يشكل حوالي 18% من بين السرطانات التي تصيب الإناث ويشكل حوالي خمس حالات الوفيات الناجمة عن أمراض السرطان حول العالم. ترتبت الدراسة الحالية على التحقق من دور بعض المعلومات السرطانية في عينات عشوائية للمرضى الوافدين إلى مستشفى الديوانية التعليمي في محافظة الديوانية . تم جمع 10 ملي لتر دم من 50 مريضة مصابة بسرطان الثدي في مرحلة ما بعد العملية الجراحية كمجموعة مرضى و 50 أنثى لا تعاني من أي نوع من أنواع السرطانات كمجموعة سيرة في الدراسة الحالية, تراوحت أعمار كلا المجموعتين بين 18 إلى 80 سنة. في مجال العلاقة بين مرض سرطان الثدي وبعض المعلومات السرطانية أظهرت نتائج دراستنا وجود علاقة إحصائية ($p<0.05$) بين الإصابة بسرطان الثدي وارتفاع تركيز CA-15.3 و CEA في مصل المرضى إلى مستويات عالية بالمقارنة مع مجموعة السيطرة $P < 0.0001$. كذلك أظهرت نتائج دراستنا عدم وجود علاقة إحصائية بين CA-15.3 و CEA $(p=0.185)$ لكن هنالك علاقة موجبة طفيفة ($r = 0.2432$).

Introduction: Breast cancer is the most common type and one of the most common cause of cancer death for women in Western countries¹. About 1.2 million women will be diagnosed with breast cancer annually worldwide and about 411.000 will die from this disease². According to American Cancer Society 95% of new breast cancer cases and 97% of breast cancer deaths occur in women aged 40 and older³. Also, 50% of women who developed breast cancer are age 61 or younger at the time of diagnosis⁴. Lifetime risk of developing this malignancy is 12.2% and a lifetime risk of death is 3.6%^{4,5}. Multiple factors are associated with an increase in breast cancer risk, including genetic and familial predisposition, hormonal factors, diet, benign breast diseases and environmental factors⁵. Over the past decade many improvements and new discoveries in the diagnosis, staging and treatment of breast cancer patients, resulted in increased survival of breast cancer patients. Breast cancer is a heterogeneous and progressive disease and its early detection remains one of the most urgent issues in cancer research⁶. Because many breast cancers still escape early detection, identification of biological tumor markers able to reveal early stage disease

may greatly reduce related mortality ⁷. Furthermore, an effective follow-up is needed for all treated patients who may develop progression recurrence of the disease during their life⁸. In parallel, the immune system is recognized as an extrinsic tumor-suppressor that can eliminate epithelial cells that have transformed to breast cancer cells and limit their growth when they have escaped intrinsic tumor suppression mechanisms ⁹. Therefore, it has been suggested that the immune system plays dual host-protective and tumor-promoting roles in breast cancer initiation and progression, by mechanisms that may shield breast cancers from immunosurveillance and enable breast cancer cells to evade immune cell induced apoptosis and produce an immunosuppressive tumor microenvironment ¹⁰. Although the measurement of tumor markers in breast cancer has been studied for nearly 20 years, their usefulness remains unclear. In patients with metastatic breast carcinoma, tumor markers appear to be useful during follow-up, but a wide range in rates of marker positivity has been reported: 50%–80% (1-3). Breast cancer is the most common malignancy in women. Successful treatment of breast cancer relies on a better understanding of the molecular mechanisms involved in breast cancer initiation and progression ¹¹. The CA 15-3 levels increase was observed in various malignant tumors, but this is a useful marker for breast cancer metastasis and is determined in monitoring disease progression and success of therapy, It is not used as screening test or as a test for primary diagnosis because it has low diagnostic sensitivity. CA 15-3 alone, however, is not recommended as a marker for either diagnosis or detection of early recurrence of breast cancer according to the American Society of Clinical Oncology (ASCO) guidelines, because of insufficient data, the ASCO also does not recommend the use of CA 15-3 alone as a marker for monitoring response. It should be noted that the elevation of CA 15-3 between 4 and 6 weeks after initiation of a new therapy, i.e. spurious early rise (surge), indicates poor prognosis¹².

Aim of Study: Study of some predisposing genes and tumor markers to reach to more frequent and dangerous factor among breast cancer patients through the following objective:

Study and measure some standers of tumor markers(CA-15.3 & CEA) which is used in identification and diagnosis of disease by using Fully-auto chemiluminescence immunoassay – CMIA

Materials and Methods

Subject : The current study was conducted on 100 females (50 patients group and 50 controls group).The patients were females who had a breast cancer (post-operative stage) . Both groups include females with 18-80 years old. The patients were referred to Al-Diwanya Teaching hospital , department of oncology, during the period March-November 2016, all patients were in the post-operative period. Demographic and risk factor data were collected using a short structured questionnaire, that included information on age, weight, height, marital status, number of pregnancies and children, age at first child birth, average lactation term, family history of breast cancer or other cancers (first degree relatives), age at menarche, age at marriage, menopausal status and age at onset. Furthermore, the patients were also followed-up after the surgical operation to define the histopathology classification of breast tumor, and on which, lymph node metastases and cancer stage at the time of testing were recorded. Another group include healthy females without any history family of breast cancer also included in this study as a control group.

Tumor marker analysis

Fully-auto chemiluminescence immunoassay – CMIA

1-Test Procedure for CEA and CA-15.3

Use an anti-CEA and ant-CA15.3 monoclonal antibodies to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control are mixed thoroughly with FITC Label and nano magnetic microbeads in a cuvette incubated at 37°C, then cycle washing adding for 1 time. Then added ABEI Label and incubated to form a sandwich, after sediment in a magnetic field, sucked the supernatant then cycle washing for the 2nd time. Subsequently, Starter1+2 substrates are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the

concentration of CEA and CA15.3 present in samples.

2-Test Procedure of CEA and CA-15.3 Tests

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the reagent Integral. refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions, procedure steps in ready kit maglomi, germany.

Calculation of Results

The analyzer automatically calculates the CEA and CA15.3 concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating 3/4 Instructions.

Statistical analysis: Statistical analysis was performed by Social Science Statistics and the Statistical Package For Social Sciences version 19 for Windows Software and Microsoft Excel 2010. Continuous random variables of age and serum concentration of immunological makers that normally distributed are described by mean, SD (standard deviation), SE (standard error), and the parametric statistical tests of significant. ANOVA test are used to analysis the statistical significance of difference in mean between more than 2 groups and when ANOVA model shows statistically significant differences, additional exploration of the statistical significance of difference in mean between each 2 groups was assessed by Bonferonni t-test .The statistical significance, direction and strength of linear correlation between 2 quantitative variables was

measured by Spearman's rank and Pearson linear correlations coefficient as in state of serum markers. Moreover measure the strength of association between 2 categorical variables, such as the presence of certain genotype and disease status the odds ratio (OR) and Chi-square (χ^2) test were used. P value calculate from different tests depend on variables and that less than the 0.05 level of significance was considered statistically significant^{13, 14}.

Results

1-The Association Between Breast Cancer And Selected Immunological Marker

Tables (1) show significant association between Immunological Marker (CA 15-3) and breast cancer ($p < 0.05$). Patients have high concentration of CA 15-3 mean (18.396) in compare with controls Mean (8.136).

Table(1): The case-control difference in mean serum concentration of tumor antigen CA 15-3

serum concentration of tumor antigen CA 15-3 (ng/ml)	Case (breast cancer)	healthy controls	P – value
Range	3.9 – 42	28 – 0.5	< 0.05
Mean	18.396	8.136	
SD	8.871	6.58	
SE	1.255	0.931	
N	50	50	

❖ Significant ($p < 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number

Tables (2) show significant association between Immunological Marker (CEA) and breast cancer ($p < 0.05$). Patients have high concentration of CEA mean (11.66) in compare with controls Mean (5.086).

Table(2): The case-control difference in mean serum concentration of CEA

Serum concentration of CEA (ng/ml)	Case (breast cancer)	Healthy controls	P – value
Range	1.9 – 24.2	0.5 – 16.5	< 0.05
Mean	11.66	5.086	
SD	5.693	4.085	
SE	0.81	0.578	
N	50	50	

❖ Significant ($p < 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number

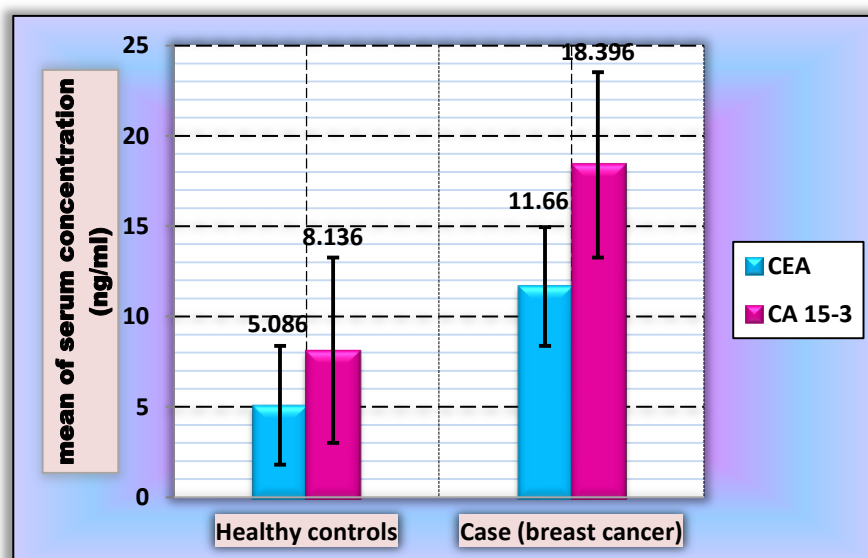


Figure (1): Bar chart show mean differences of CA 15-3 and CEA in patient and control

2-Correlation Between CA 15-3 and CEA In Breast Cancer Patients

In our present study we try to found relationship between both tumor markers which included CEA and CA.15.3, Figure (1) show not significant association between CA 15-3 and CEA in breast cancer patients ($P=0.185$), but moderate positive correlation is appeared ($r = 0.2432$).

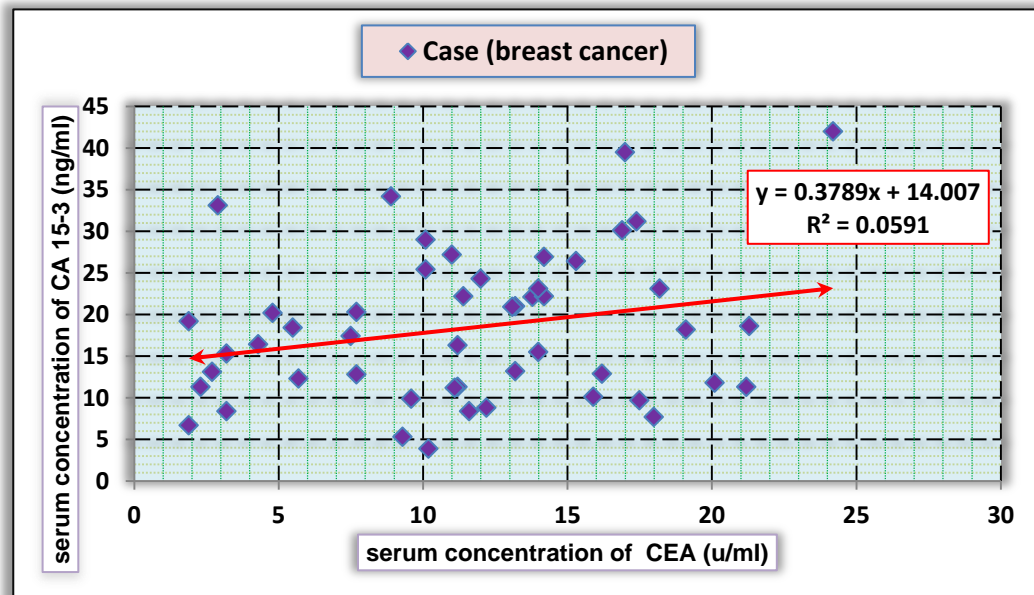


Figure (2): Scatter diagram showing the linear correlation between serum concentration of tumor antigen CA 15-3 and CEA ($r= 0.2432$, $p= 0.185$) in tumor patients

Discussion

1-The Association Between Breast Cancer And Selected Immunological Markers

Present study include two tumor markers are cancer antigen 15.3(CA-15.3) and cytoembryonic antigen (CEA) measured in 50 females with breast cancer as a patients group and 50 healthy females as a control group. The results of present study show significant association between serum concentration of CA-15.3 and breast cancer disease ($p<0.05$), Patients have high concentration of CA 15-3 (mean 18.396) and this result considered as a high ratio in compare with levels of control

group (mean 8.136) this results agreed with ¹⁵ who found significant association of 150 breast cancer patients(mean 16.94) in compared with for control group (mean 7.21). So our present results matched with results stated by ¹⁶ who found serum CA-15.3 of 100 females with breast cancer have significant association with 100 females in control group ($p<0.0001$).

The results in present study show a significant association between elevation CEA and breast cancer disease ($p<0.05$), breast cancer patients have a elevated concentration of CEA (mean 11.66) in compare with control group (mean 5.086), this result agreed with results of ¹⁷ who found a significantly association in levels of CEA between breast cancer patients group and controls group ($p<0.05$), and so who found elevated concentration of CEA in 140 post-operative breast cancer as a patients group whom visited most China hospitals (mean 13.54) compared with 280 females as a control group (mean 3.87), the results of our present study so agreed with ¹⁸ and ¹⁹ who did a comparison between post-operative breast cancer females as a patients group and healthy females as a control group, and who found the levels of CEA in patients serum elevated to highest levels (means 16.43 and 19.08 respectively) compared with controls (mean 4.03 and 6.91 respectively), and appeared a significant association between both groups ($p<0.05$).The utility of these serum biomarkers may be served as effective prognostic indicators for post-operative breast cancer patients, further researches are needed to determine the effectiveness of these serum biomarkers in formulating treatment strategies in clinical practice, therefore, CEA levels greater than $7.5 \mu\text{g/L}$ are associated with high probability of subclinical metastases ²⁰.Prognosis of patients whose CEA level was within the normal range at the time of diagnosis is significantly better than those with elevated CEA levels ²¹.

Baseline CA 15-3 might be value in the identification of higher risk of relapse, where adjuvant chemotherapy must be introduced. In other words an explicitly that presence of an abnormal CA 15-3 pre-surgical value is associated with an increased risk of recurrence and death ²².The prognostic value of preoperative serum tumor markers CEA and CA 15–3 was evaluated in our present study including 50 patients compared with low levels in 50 females among control group, the results showed

that preoperative serum CEA and CA15-3 levels were independent factors affecting prognosis. The utility of measuring CEA and CA15-3 levels in patients with breast cancer remains controversial. Due to their low sensitivity, they cannot be recommended for screening or early diagnosis, but serial levels may be useful in the early diagnosis of distant metastases, European Group on Tumor Markers has recommended the CEA and CA15-3 levels be used for assessing prognosis, the early detection of disease progression, and treatment monitoring in breast cancer markers should be measured prior to every chemotherapy course and at least every 3 months for patients receiving hormone therapy ²³.

The American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) guidelines do not currently recommend the use of serum CA 15-3 and CEA for breast cancer screening and directing treatment ²⁴. On the one hand, this may partly due to the conflicting conclusions of different researches ²⁵. CA 15-3 increase of 5-10 times above normal upper limit can predicts breast cancer, however, a low value cannot exclude metastasis making, CA 15-3 more of prognostic rather than diagnostic marker ²⁶. On the other hand, the low positive rate of serum tumor markers is also the possible reason, The incidence of breast cancer has been steadily increasing in the last two decades, however, due to the early detection and increased use of more effective systemic therapy, the survival rates of breast cancer have improved in recent years, and early breast cancer accounted for a large proportion. Previous researches demonstrated that the CEA and CA15-3 levels are associated with tumor burden indicators including tumor size and lymph node status and patients with locally advanced breast cancer exhibit significantly higher levels of CEA and CA-15.3²⁷. The sensitivity of tumor markers is significantly higher in patients with advanced disease, and is related to the site of recurrence ²⁵. CA 15.3 and CEA are not useful in the early diagnosis of loco regional recurrence, for which clinical examination is superior. However, abnormal CEA and CA 15.3 levels are founding 40–50 and 50–70% of patients with distant metastases²⁸. Since elevated levels of CA-15.3 and CEA are related to the tumor burden and higher levels may indicate an increased likelihood of systemic metastases Studies by Lee et.al showed that elevated tumor marker levels are more frequently observed in metastatic breast

cancer patients than in primary breast cancer, and patients who had elevated tumor marker levels before surgery also showed more frequent elevation at recurrence ²². Since markers are relatively easy and inexpensive to measure, regular measurement of serum tumor marker levels could provide useful information for earlier detection of recurrence ²⁹.

2-Correlation Between CA 15-3 and CEA In Breast Cancer Patients

In field of relationship between both tumor markers among our study ,our results show there are no relationship and association in nature and work between cancer antigen 15.3 and cytoempryonic antigen through the levels of this markers in serums of breast cancer patients , and the statistical results in this study show no significant association between CA 15-3 and CEA in patients group ($p= 0.185$) , but moderate positive correlations is appeared ($r = 0.2432$), this result agreed with ³⁰ who study on 70 Iranian women who observed a significant increased value of CA15-3 and CEA in patients group of breast cancer females as compared with healthy control females and no significant association between both biomarkers among patients group ($p= 0.112$). And as well agreed with ³¹ who study on 150 females with breast cancers who visited Ramses Medical Center in Narew and reached to no linear correlation was found between both CEA and CA15-3 and the other variables which showed a mild ($R=0.57$) linear relationship , Moreover, no linear correlation was found between the values of CA15-3and CEA ($R=0.29$) and no significant association ($P=134$). As well as our results matched with ³² who showed did not identify significant differences in CA 15-3 and CEA levels between different sites of metastasis in patients with breast cancer.CA 15-3 in combination with CEA is also relevant tumor markers in breast cancer , and the serum concentration of marker CA 15-3 has superior prognostic relevance in relation to CEA, but unlike these authors, ³³ reported that prognostic value of CEA is higher than that of CA 15-3, which demonstrated that this marker has conflicting implications in breast carcinogenesis. ³⁴ measurement of tumor markers is a tool for detection of distant metastases, and the marker CA 15- 3 seems more efficient when compared to CEA and no linear relation between it . Monitoring of breast cancer patients after surgical treatment using only

this tumor markers is insufficient, however, simultaneous use of both serum markers (CA 15-3 and CEA) allows the early diagnosis of metastasis in up to 60–80% of patients with breast cancer ³⁵. A better understanding of the correlation between serum CA 15-3 and CEA levels and the intrinsic biological characteristics of breast cancer may affect the selection of adjuvant therapy, the combined measurement of both serum markers allows early diagnosis of metastases in up to 60-80% of breast cancer patients ³⁶. Elevated serum CA 15-3 and CEA levels at recurrence suggest increased tumor burden and may be prognostic for survival for metastatic breast cancer patients ³⁷.

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