Original Article

Association of Interleukin-12 and Carbohydrate Antigen 15.3/Carcinoembryonic Antigen in Progression of Breast Cancer in Al-Najaf Province

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Abstract

Background: Breast cancer (BC) is one of the top cancer-related mortality in women; cytokines is a key player in promoting or inhibiting cancer. Reliable indicators for early diagnosis and crucial for enhancing breast cancer (BC) prognosis. **Objectives:** This search design to evaluate interleukin-12 (IL-12) concentration as novel biomarker complementing CA-15.3 (carbohydrate antigen 15.3) and CEA (carcinoembryonic antigen) for BC diagnosis and metastasis prediction. **Materials and Methods:** A total of 130 subjects including 90 breast cancer women attended the Middle Euphrates Cancer Center in Al-Najaf province during 16-8-2022 to 20-12-2022 and 40 healthy women as a case control study. Blood sample was collected from all subjects. IL-12 was determined in serum by enzyme-linked immunosorbent assay, and CA-15.3/CEA was determined in serum by electrochemiluminescence immunoassays (Cobas E411). **Results:** The results showed a significant increase in serum concentration of CA-15.3/CEA in BC patients women (27.1 ± 10.6, 8.8 ± 2.6, respectively) compared to healthy subjects (9.2 ± 3.5, 2.3 ± 1.0, respectively). According to the stage of BC, the concentration of CA-15.3 and CEA increased with the severity of the disease and this is considered as biomarker for the progressive state. While the mean levels of serum IL-12 in healthy women were higher than women with BC (46.3 ± 15.2 vs. 25.56 ± 11.4) pg/mL, the difference was highly significant ($P < 0.001$), and according to BC stage, IL-12 serum level decreases with disease progression, so IL-12 in stage I is lower than healthy group and higher than stage IV. These results indicate there is a negative relationship between CA-15.3/CEA and IL-12. **Conclusion:** Serum levels of IL-12 were significantly decreased in BC Iraqi women and decreased correlation with progressive of disease may be considered as prognostic factors for disease outcome and CA-15.3 and CEA highly increased in BC patients in comparison with controls and used as an indicator for helping clinicians to assess course of therapy and disease progression and is considered as prognostic biomarker.

Keywords: Breast cancer, CA-15.3, CEA, IL-12

Introduction

Breast cancer (BC) is the most common type of cancer among women, accounting for 25% of all women diagnosed with cancer and 15% of all female cancer deaths with more than 2 million new cases globally each year.[1] In Iraq, BC ranks first among malignancies among Iraqi women and the second leading cause of death for women. The latest annual report of the Iraqi Cancer Registry for 2018 estimated that 38 million Iraqis, including (31,502) new cancer cases and 10,293 deaths.[2] Metastasis of malignant tumors is the cause of over 90% of cancer-related deaths.[3]

Tumor markers are a class of active substances produced by the interaction between host and tumor, which indicates tumor presence and growth. At any stage, the concentration of tumor markers is determined by several tumor parameters, such as size, mass, degree of expression, catabolism, secretion rates, and drug resistance.[4]

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_563_23

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Submission: 15-May-2023 **Accepted:** 12-Jun-2023 **Published:** 23-Jan-2026

How to cite this article: Saeed AZ, Darweesh MF. Association of interleukin-12 and carbohydrate antigen 15.3/carcinoembryonic antigen in progression of breast cancer in Al-Najaf Province. Med J Babylon 2025;22:1117-22.

CA-15.3 is a large transmembrane glycoprotein (produced from Mucin 1 gene) that can be found in all epithelial organ cells, including those in the breast, colon, ovary, and pancreas.[5] It is located on the surface of BC cells and sheds into bloodstream as antigens. The decrease in CA15.3 levels indicates good medication therapy effect or stable disease, while elevated concentration indicated disease progression, recurrent BC, and metastatic conditions of BC.[6]

Cytokines are highly stimulatory secretory proteins that facilitate communication between cells in the immune system. In recent decades, much attention has been focused on the role of cytokines in BC. Some cytokines stimulate BC proliferation and others modulate the antitumor response, and concentrations of cytokines can be used as a prognostic marker for cancer.[7] Interleukin-12 (IL-12) plays a key role in shaping the development of antitumor or protumor immunity by inducing T-cell differentiation and activating natural killer cells, it enhances the production of interferon-γ by T and natural killer cells.[8]

Materials and Methods

Patients and control characterization

A case-control study included 90 BC women with age range from 29 to 72 years with different stages of BC collected from the Middle Euphrates Cancer Center in Al-Najaf province from August till December 2022. Clinical stage, tumor size, lymph node metastasis, distant organs metastasis, and grade were diagnosed by oncology specialist. And 40 healthy women with age range between 27 and 69 years.

Sample collection

About 3 mL blood was transferred to a serum separation gel tube and left for 30 min at room temperature for clotting and then centrifuged at 4000 rpm for 5 min and then stored at −20°C until used for IL-12 measurement according to the company (Elabscience, Houston, Texas, USA) and CA-15.3/carcinoembryonic antigen (CEA) protocol according to Cobas E411 protocol.

Ethical Approval

The study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki. It was performed with patients' consent both verbally and analytically before sampling. The study protocol, subject information, and consent form were reviewed and approved by the local ethics committee according to document number 8356 in 11/09/2022 to get this approval.

Statistical analysis

Statistical analysis was carried out by using statistical software (IBM SPSS Statistics 26). The quantitative data were given as mean ± SE and the differences were tested by *t*-test (two means) and one-way ANOVA test (more than two means). Probability ($P \leq 0.05$) that was measured was statistically significant.

Results

The findings showed that patients ranged in age from 29 to 72, with a mean age of 54.9 (16) years; 43 (47.7%) patients were under 50, while 47 (52.2%) were above 50. Also, 81 (90%) patients were married and 19 (10%) were single. About 38 (42.3%) were with family history and the remaining 52 (57.7%) were without family history.

In addition, for 40 healthy women, the age range was 27–69 with mean age 51.88 ± 14.7 years, from which 18 (45%) were less than 50 years and 22 (55%) were more than 50 years, also 29 (72.5%) healthy women were married.

Table 1: Case–control comparison

	Case-control comparison		
Age (years)	**Breast cancer patients**	**Healthy controls**	***P***
Range	29–72	27–69	0.83
Mean ± SE	54.9 ± 16	51.88 ± 14.7	[NS]
N	90	40	
≥50 years	47 (52.2%)	22 (55%)	
≤50 years	43 (47.7%)	18 (45%)	
Married	81 (90%)	29 (72.5%)	
Family history	38 (42.3%)		
Stage I	4 (4.4%)		
Stage II	34 (37.7%)		
Stage III	26 (28.8%)		
Stage IV	26 (28.8%)		
Nonmetastatic BC	64 (71.1%)		
Metastatic BC	26 (28.8%)		

NS = no significant, SE = standard error, *N* = number

The current study shows that of 90 patients, 26 (28.8%) patients had metastatic BC and were significantly lower than nonmetastatic BC 64 (71.1%).

And according to tumor-lymph node-metastasis (TNM) staging system, this study reveals that stage II had the highest percentage 34 (37.7%) followed by stage III and stage IV 26 (28.8%) and the lowest percent for stage I 4 (4.4%), as shown in Table 1.

Evaluation CA-15.3 and CEA concentration in BC patients and controls

The present study illustrated that significantly increased ($P < 0.005$) in serum concentration CA-15.3/CEA in BC patients women (27.1±10.6, 8.8±2.6, respectively) compared to healthy group (9.2±3.5, 2.3±1.0, respectively) as shown in Figure 1, also, the concentration of CA-15.3/CEA is elevated in patients with metastatic BC, while its level is rarely increased in patients with localized cancer as shown in Figure 2A. The average concentration of CA-15.3 in the fourth stage was found to be extremely high (46.57 pg/mL) compared to the average concentration in the first stage (14.58 pg/mL), and the average concentration of CEA in the fourth stage was found to be lower (13.5 pg/mL) than the average concentration in the first stage (4.6 pg/mL) [Figure 2B].

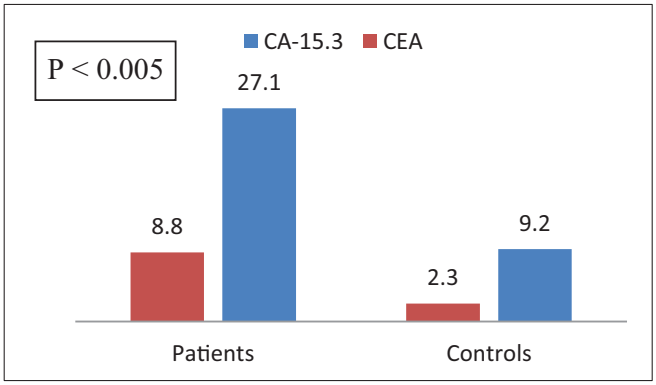

Figure 1: A comparison between patient group and control group in the mean of serum concentration of CA-15.3/CEA

Evaluation of serum IL-12 level in breast cancer patients and controls

A comparison of IL-12 serum level between patients with BC and control groups was carried out and the results show that the mean levels of serum IL-12 in BC patients less than healthy women (46.3 vs. 25.56) pg/mL, the difference was highly significant ($P < 0.001$). And the mean levels of serum IL-12 decrease with the progression of the disease, the results show the level of serum IL-12 in stage I was higher than stage IV and in metastatic BC it was lower than nonmetastatic BC healthy women as shown in Figures 3 and 4.

Our results indicate there is negative relationship between CA-15.3/CEA and IL-12 as shown in Table 2.

Discussion

These results illustrated that aged patients were with more incidence than young patients; this consist with the local study,[9] they found that risk for BC associated with increased age especially ≥50 years, older age has been exposed to more cancer-causing substances for a longer period of time or the age developed gives the cells more time to change mutation to grow into cancer. In another local study, Abbas[6] found that 70% of females with BC aged more than 50 years. Hantoosh[10] discovered that the average patient age was 49.66±12.50 years, and that 44% of patients were over 50 years old. Also, Dogra *et al.*[11] discovered an intriguing link between aging and the development of cancer due to changes in cellular and tissue structure and explained that age is considered the strongest demographic risk factor for most chronic diseases, including cancer.

A local study[12] showed that 91 (90.10%) of BC patients were married, 4 (3.96%) were single, 1 (0.99) was divorced, and 5 (4.95) were widowed that agree with our results. Another local study consistent with our findings showed that BC is common in married women, with about 77.5% of cancers found in married women, while 22.5% were

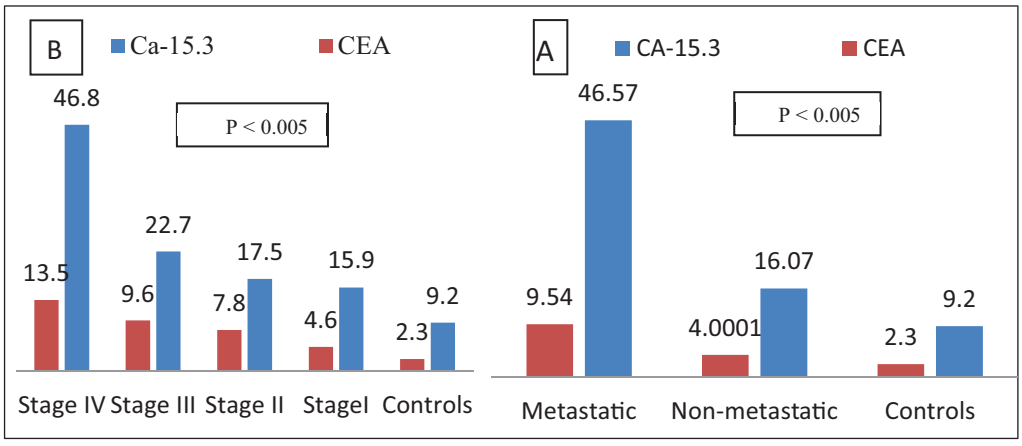

Figure 2: CA-15.3 and CEA serum concentration in Breast cancer patients with related to (A)metastatic and (B) stages compared to control

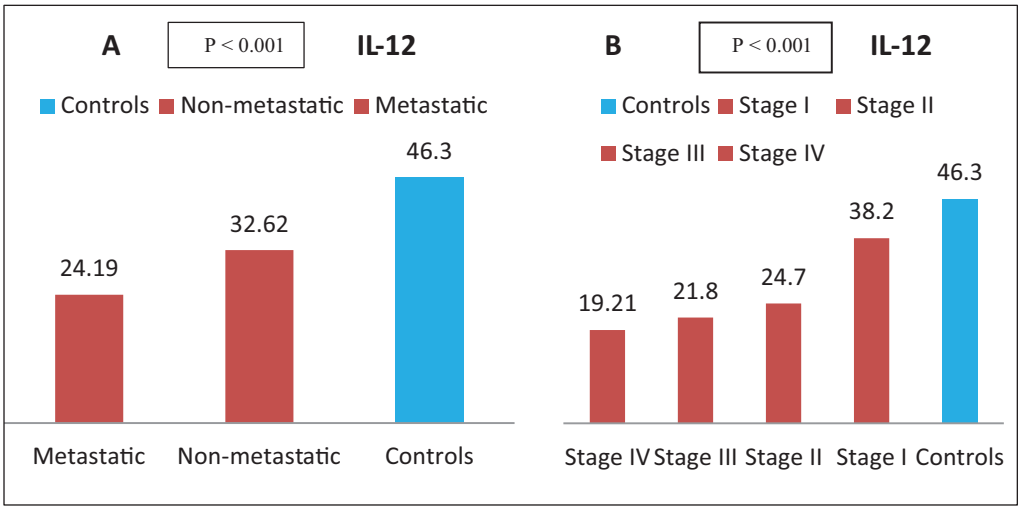

Figure 4: IL-12 serum level in BC patients with related to (A) metastatic and (B) nonmetastatic stages and control

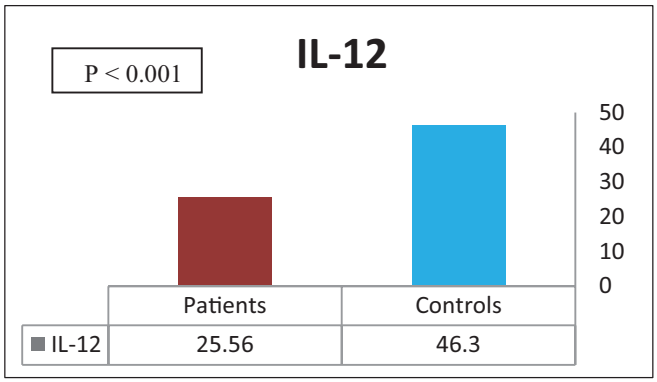

Figure 3: The mean of serum level of IL-12 in BC patients and controls

found in unmarried women.[13] Our results were similar to study that revealed a comparison between married and unmarried cases, where married cases contribute a higher percentage than unmarried women (97.1% and 2.9%, respectively), and they confirmed that married women are more susceptible to hormonal change.[14]

Our results agree with a local study[10] who found a strong risk with respect to family history of BC and showed that 7 (14%) of the BC patients had a positive family history of BC, and these findings were significantly associated with the risk of BC when compared to the healthy control. Of all 315 women with metastatic BC, only 19 (6%) had a positive family history of BC or other types of cancer. The remainder was negative or unknown family history.[15]

According to the TNM staging system, stages I, II, III, and IV study was documented locally in which 57 (9.5%), 284 (47.1%), 200 (33.2%), and 55 (9.1%) patients, respectively, were stage II and got the highest percentage.[16]

In Basrah, Touma and Shani[17] revealed that of 35 patients, 14 (40%) had metastatic BC and 21(60%) were without metastatic BC. In another local study, Mutar *et al.* (2019) observed that 25% of patients show metastatic BC and 75% show nonmetastatic BC.[18]

The BC follow-up examines the blood markers CEA and MUC1 (CA-15.3), which are beneficial and more successful in managing the condition.[19]

Significantly higher levels of CEA or CA-15.3 have been shown in patients with late-stage BC than in those with early disease.[20]

In the same line, Iraqi[21] confirmed that CA-15.3 remains the most frequently used tumor marker in BC, and its clinical value is attributed to the association of CA-15.3 with tumor burden, such as tumor size and lymph node status. Furthermore, a study noted that an elevated CA-15.3 level was more frequent in patients with metastatic BC than in early breast cancer.

Another local study[6] revealed that the serum cancer antigen 15.3 concentration was high significantly increased ($P \leq 0.01$) as compared to control groups (23.17 ± 0.78 U/mL versus 9.15 ± 0.70 U/mL), respectively, which found the CA-15.3 median level of BC group was 2.36 times higher compared to the control group.

Hasan[22] observed higher CA-15.3 compared to CEA in a pretreatment cohort of BC patients compared to healthy controls and found that late-stage patients showed higher positive serum levels compared to early stage one for both markers with CA-15.3 being preferred over CEA. Also, our study confirm with Gaughran *et al.*[23] that observed CA-15.3 and CEA elevated significantly in BC patients compared to the evaluation of IL-12 levels in breast cancer patients and controls.

A local study[24] notes that the serum level of IL-12 in benign and malignant patients was significantly ($P < 0.05$) decreased when compared to controls (82.88 ± 8.07 vs. 173.5 ± 28.34 pg/ml) and (24.41 ± 1.55 vs. 173.5 ± 28.34 pg/mL). These results are consistent with a study finding that suggested decreased IL-12 production may contribute to

Table 2: Comparison between mean of IL-12 serum level and CA-15.3/CEA tumor markers

Breast cancer patients	IL-12	CEA	CA-15.3
	Mean ± SE	Mean ± SE	Mean ± SE
All patients	25.56 ± 11.4	8.8 ± 2.6	27.1 ± 10.6
Stage I	38.2 ± 13.6	4.6 ± 09.3	15.9 ± 2.8
Stage II	24.7 ± 12.4	7.8 ± 04.8	17.5 ± 5.3
Stage III	21.8 ± 9.2	9.6 ± 1.5	22.7 ± 8.0
Stage IV	19.21 ± 8.8	13.5 ± 2.4	46.8 ± 12.6
Nonmetastatic BC	32.62 ± 14.5	4.01 ± 2.33	16.07 ± 6.90
Metastatic BC	24.19 ± 11.7	9.54 ± 4.38	46.57 ± 12.66

the growth and development of tumors.[25] Additionally, according to Ali,[26] patients with lower serum levels of IL-12B may have the AA genotype, which results in less IL-12 production and makes them more prone to developing progressive illness. As tumor grades increased, serum levels of IL-12 declined.[27]

When compared to the healthy group, the levels of IL-12 in stage I BC patients were significantly lower. This result indicates that decreased IL-12 production may contribute to the early stages of tumor formation and progression.[28] Al-Sherify *et al.*[29] illustrated that IL-12 mean serum level decreased to 33.9 ± 9.9 pg/mL in brain tumor patients compared to level in controls (47.68 ± 3.5 pg/ mL). Also, there was a higher reduction in late stages of in gliomas, meningioma, and schwannoma (30.55 ± 10.8, 37.6 ± 8.4, and 33.8 ± 8.7 pg/mL, respectively).

Serum CEA and CA-15.3 levels were statistically significantly lower in control (C) than both benign (B) and BC; on the contrary, IL-12 serum level was significantly higher in control compared to BC and benign tumor patients.

The serum levels of CEA and CA-15.3 were statistically lower in control than in both benign BC and malignant BC; on the contrary, the level of serum IL-12 was significantly higher in control than in BC and benign tumor patients.[30-32]

Conclusion

Serum levels of IL-12 were significantly decreased in BC Iraqi women and this decreased correlated with progressive of disease that may be considered as prognostic factors for disease outcome and CA-15.3 and CEA highly increased in BC patients in comparison with controls and used as an indicator for helping clinicians to assess course of therapy and disease progression and is considered as prognostic biomarker.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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