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Clinical Significance of Serum Cancer Antigen 15-3 (CA15-3) as Prognostic Parameter in Non-metastatic Breast Cancer Patients: is still a valid test?

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ABSTRACT

Background: Recently, the predictive usefulness of variations in cancer antigen CA15-3 for breast cancer (BC) has received much attention.

Objective: This study aimed to assess the relationship between the kinetics of the serum level of CA15-3 and the outcome of patients with non-metastatic BC.

Patients and methods: This prospective single center trial was carried out in Medical Oncology Department, South Egypt Cancer Institute (SECI), Assiut University, on 75 female patients with non-metastatic BC patients. The serum level of CA15-3 was evaluated via Roche Elecsys at three time points (pre-operative, post-operative, and after one year of follow up).

Results: Serum CA15-3 concentration showed a significant reduction from pre- to post-operative time, from pre- to after one year of follow up and from post-operative time to after one year of follow up ($P< 0.001$). No Significant relation was observed between CA15-3 and the clinic-pathological data of the studied non-metastatic BC patients. Also, CA15-3 level had no role in the survival outcome of the studied cases.

Conclusion: The finding of the current study indicated that it is not necessary to mutually examine the pre- and post-operative serum level CA15-3 to evaluate its role as this will not add much more clinical benefit, and that we culd examine the pre-operative serum CA15-3 concentration to avoid wasting time, effort and money.

Keywords: Breast cancer, Metastasis, Cancer antigen (CA) 15-3.

INTRODUCTION

As the most prevalent cancer in women, BC is a major health problem with over a million new cases identified globally each year [(1)].

Because of extensive research on the biology and behavior of BC, survival rates have increased despite the disease's rising incidence. On the other hand, patients' QoL and survival rate are severely impacted by therapy failure. In order to improve prognosis, it is crucial to establish trustworthy prognostic indicators to guide treatment decisions for BC [(2)]. Both healthy and malignant cells have the ability to emit a chemical known as a tumor marker. When cancer progresses, these levels are significantly greater. In order to determine the need for additional management modifications, tumor markers would be a good starting point for evaluating the prognosis of cancer after chemotherapy and radiation [(3)].

The MUC-1 gene produces Cancer Antigen 15-3 (CA15-3), and mucins are abnormally overexpressed in many adenocarcinomas in an underglycosylated form that is subsequently released into the circulation. It has been demonstrated that in patients with advanced BC, CA15-3 is a robust prognostic indicator and an independent predictor of the first recurrence. In BC, a high pre-operative CA15-3 level is positively correlated with both the tumor burden and independent prognostic factors. It has the potential to be applied in clinical contexts to forecast patient outcomes and suggest adjuvant therapies for better results [(4)]. This study aimed to assess the relationship between the serum level of CA15-3 and the outcome of patients with non-metastatic BC.

PATIENTS AND METHODS

Patients: This prospective hospital-based cohort study was conducted over a period of one year from the first of February 2022 up to the end of January 2023. The study included 75 adult females with non-metastatic BC who attended to the Medical Oncology Department, South Egypt Cancer Institute, Assiut University, Egypt.

Inclusion criteria: Adult female patients with non-metastatic BC; CA15-3 level was determined before surgery. Complete results of estrogen receptor (ER), progesterone receptor (PR), HER2, Ki-67, and histologic grade were assessed.

Exclusion criteria: Metastatic BC, carcinoma in situ, pregnant or lactating women, and those with other malignant tumors.

TNM staging was determined using the Eighth Edition American Joint Committee on Cancer guidelines [(5)]. Tumors that contained more than 1% nuclear-stained cells were considered ER and PR positive. A 3+ or 2+ immunohistochemistry score indicated HER2-positivity, which was verified by a HER2-specific fluorescence in situ hybridization (FISH) test. Ki-67 staining was performed using a cut-off value of 14%. According to the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer [(6)]. The molecular

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subtypes were classified into four groups: Luminal A (ER+ and/or PR+, HER2-, Ki-67 <14%), Luminal B (ER+ and/or PR+, HER2+ and/or Ki-67 ≥14%), HER2 positive (ER- and PR-, HER2+), and triple-negative (ER- and PR-, HER2-).

The data regarding demographics, complete clinical characteristics, pathological characteristics, stage determination, and complete pathologic response were obtained from our medical records. Additionally, laboratory tests were performed to determine the calcium level, liver and renal function, and total blood count. For every BC patient under study, imaging tests (bone scans, breast and abdominopelvic ultrasonography, and chest radiography) were carried out. Medical imaging techniques were used to confirm distant metastases, and pathology evaluation was performed if necessary.

Sample Collection and tumor marker analysis:

Every serum sample was taken in the early hours of the day. An hour after collection, blood taken without anticoagulant was centrifuged at 1600 ×g for 10 minutes at 4°C. The blood was then put into tubes and stored at -80°C for future research. Using an automated electrochemistry luminescence immunoassay system (Roche Elecsys), the blood level of CA15-3 was assessed three times (pre-operative, post-operative, and one year after the procedure). The marker's result was interpreted as positive or negative based on whether the amount was above or below 25 U/mL cut-off value for CA15-3.

Ethical approval: This study was approved by The South Egypt Cancer Institute Medical Ethics Committee (IRB number 584). Following receipt of all information, signed consent was provided by each participant. The Helsinki Declaration was adhered to at every stage of the investigation.

Statistical analysis

SPSS version 22.0 was used for all statistics. When applicable, percentages (number of instances) and frequency distributions (percentages) were used to statistically describe the data along with means, standard deviations (SD) and median (range) for quantitative data and compared by Mann Whitney U test. Wilcoxon sign test was used for comparing the quantitative data overtime. P-value set significant at ≤ 0.05 level.

RESULTS

CA15-3 level in different time points: By comparing each successive time points we observed that: **from pre- to post-operative time,** CA15-3 showed significant reduction (from 15.70 ± 6.98 to 13.91 ± 6.11, P<0.001) respectively. While, it showed significant increase **from pre- and post-operative time to after one year of follow up,** (from 15.70 ± 6.98 to 23.15 ± 9.99, P<0.001, and from 13.91 ± 6.11 to 23.15 ± 9.99, P<0.001) respectively as shown in table (1).

Table (1): The level of CA15-3 biomarker among the studied BC cases over the studied time points (pre-operative, post-operative, after one year of follow up)

	CA15-3					
	Pre-operative	**Post-operative**	**After 1 year FU**	**P value[1]**	**P value[2]**	**P value[3]**
Mean±SD	15.70±6.98	13.91±6.11	23.15±9.99	**<0.001**	**<0.001**	**<0.001**

CA15-3: Cancer antigen 15-3. Data are presented as mean ± SD. Significance defined by p<0.05.
P value[1]: for comparing pre and post-operative data.
P value[2]: for comparing pre and data after one year of follow up.
P value[3]: for comparing post-operative data and data after one year of follow up.

Level of CA15-3 according to the development of metastasis:

Table (2) showed that during the follow up period 11/75 BC cases (14.7%) developed metastasis. Based on this finding we divided the studied cases into two groups [non-metastatic, n=64, and metastatic group, n=11], and compared the levels of both studied biomarkers between them.

At the pre- and post-operative time, the CA15-3 level was comparable between metastatic and non-metastatic cases with no significant difference between them (P=0.325, and 0.341, respectively).

While, **after one year of follow up,** CA15-3 level was significantly higher among metastatic BC cases compared to non-metastatic BC cases (P=0.003).

Table (2): The level of CA15-3 among the studied BC cases according to the development of metastasis (n=75)

CA15-3	**No mets (n=64)**	**Mets (n=11)**	**P value**
Pre-operative			
Mean ± SD	15.39 ± 3.01	17.45 ± 4.85	0.325
Post-operative			
Mean ± SD	13.60 ± 3.85	15.73 ± 3.49	0.341
After 1 year of FU			
Mean ± SD	21.55 ± 5.81	32.45 ± 1.74	**0.003**

CA15-3: Cancer antigen 15-3. Data are presented as mean ± SD. Significance defined by p<0.05.

Levels of CA15-3 according to metastasis at different time points: Table (3) showed that **among non-metastatic cases**, significant reduction was observed from the pre-operative time to the post-operative time (P=0.001), and significant increase from the pre- and post-operative time to after one year of follow up (P<0.001).

While, among metastatic cases, no difference was observed in CA15-3 from pre- to post-operative time (P=0.134). While, significant increase in CA15-3 was observed from the pre- and post-operative time to after one year of follow up (P=0.006).

Table (3): The level of CA15-3 among the studied BC cases over the studied time points (pre-operative, post-operative, after one year of follow up) according to the metastatic status

	CA15-3					
Variable name	**Pre-operative**	**Post-operative**	**After 1 year of FU**	**P value[1]**	**P value[2]**	**P value[3]**
Non metastatic (n=64)				**0.001**	**<0.001**	**<0.001**
• Mean±SD	15.39±7.01	13.60±5.85	21.55±8.81			
Metastatic (n=11)				0.134	**0.006**	**0.006**
• Mean±SD	17.45±6.85	15.73±7.49	32.45±11.74			

CA15-3: Cancer antigen 15-3. Quantitative data are presented as mean ± SD. Significance defined by $p < 0.05$.
P value[1]: for comparing Pre and post-operative data.
P value[2]: for comparing Pre and data after one year of follow up.
P value[3]: for comparing post-operative data and data after one year of follow up.

Relation between CA15-3 and the clinico-pathological details of studied BC cases: No significant association was observed between the level of CA15-3 biomarker and the clinico-pathological details of studied BC patients (P>0.05, for all), as shown in (Table 4).

Table (4): Relation between CA15-3 and the clinico-pathological details of studied BC patients (n=75)

	CA15-3, Median (range)	**P value**
Tumor size (cm)		0.335
• < 2 cm	13.3 (4.0 - 23.0)	
• 2-5 cm	14.0 (4.0 - 34.0)	
• > 5 cm	18.0 (5.0 - 29.0)	
N staging		0.742
• Negative	15.0 (4.0 - 23.0)	
• Positive	14.0 (4.0 - 34.0)	
Estrogen receptors		0.293
• Negative	13.0 (4.0 - 34.0)	
• Positive	14.0 (4.0 - 29.0)	
Progesterone receptors		0.286
• Negative	16.0 (4.0 - 34.0)	
• Positive	13.0 (4.0 - 29.0)	
HER2-neu receptors		0.705
• Negative	14.0 (4.0 - 34.0)	
• Positive	16.0 (6.0 - 27.0)	
Ki67		0.896
• Low (0 – 15%)	14.0 (4.0 - 28.0)	
• Intermediate (16 – 30%)	14.0 (5.0 - 34.0)	
• High (>30%)	13.0 (4.0 - 29.0)	
Biological subtypes		0.938
• Luminal A	14.0 (8.0 - 28.0)	
• Luminal B (HER2-positive)	16.0 (6.0 - 27.0)	
• Luminal B (HER2-negative)	13.5 (4.0 - 29.0)	
• HER2-positive	14.0 (9.0 - 26.0)	
• Triple Negative Disease	13.0 (4.0 - 34.0)	
Lympho-vascular invasion		0.534
• Yes	16.0 (4.0 - 29.0)	
• No	14.0 (4.0 - 34.0)	
Perineural invasion		0.305
• Yes	13.0 (4.0 - 29.0)	
• No	14.0 (4.0 - 34.0)	
Clinical stages		0.333
• Stage 1	11.0 (8.0 - 23.0)	
• Stage 2	13.5 (4.0 - 34.0)	
• Stage 3	16.0 (5.0 - 29.0)	

CA15-3: Cancer antigen 15-3. Data are presented as median (range). Significance defined by $p < 0.05$.

Figure (1) showed that at 12 months of follow-up, disease free was 90.5% in patients with CA15-3 < 25 U/ml, versus 77.8% in patients with CA15.3 ≥ 25 U/ml (P= 0.627).

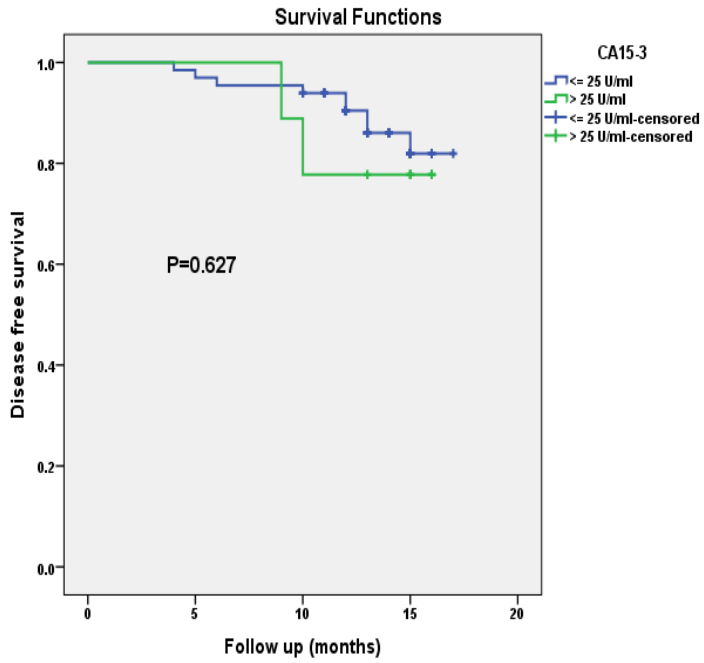

Figure (1): Disease free survival curves according to CA15-3 level.

DISCUSSION

Currently, the primary means of identifying BC metastases are imaging tests and pathology, which are not relevant to metastatic BC (MBC) screening. For many cancers, tumor markers are crucial to the diagnosis, follow-up, and prognosis [(7)]. Carcinoembryonic antigen (CEA), CA15-3, CA125, and tissue polypeptide-specific antigens are commonly utilized clinical tumor markers for BC. The majority of research focuses on the function of CA15-3 and CEA in the diagnosis of MBC [(7)]. These biomarkers are extensively utilized in the diagnosis, treatment monitoring, and prognosis of BC and are often employed as tumor markers in BC [(8–12)].

The macromolecular glycoprotein antigen known as serum CA15-3 is mostly found on the luminal side of healthy mammary epithelium, and as cells develop into malignant ones, their quantity in serum rises dramatically. Regarding the use of CA15-3 as a diagnostic tumor marker, opinions differ. Some people don't think it's very useful for detecting BC, especially in the early stages of the illness [(13)]. According to others, it has excellent clinical utility for the assessment and prognosis of BC and is a sensitive marker for BC [(4, 14, 15)]. Tumor size, clinical stage, receptor expression, metastasis, and recurrence are all associated with its level. Its accuracy and specificity are still insufficient [(16)]. According to previous literature, CA15-3 could be used as a biomarker to help diagnose cases of BC recurrence. For instance, **Shao *et al.*** [(2)] reported that in patients with metastatic BC, the preoperative blood levels of CEA and CA 15-3 are independent prognostic indicators. In order to ascertain the optimal period for assessing the blood level of this biomarker, the primary goal of the current study was to evaluate the association between the outcome of patients with non-metastatic BC and the level of CA15-3.

In the current study we examine the serum CA15-3 concentrations among the studied BC cases at three time points (pre-operative, post-operative and after one year of follow up). An interesting finding observed in the current study was that the serum CA15-3 concentration among non-metastatic cases showed significant reduction from the pre-operative time to the post-operative time, and then significant increase from the pre- and post-operative time to after one year of follow up. While, among metastatic cases; no difference was observed in CA15-3 from pre- to post-operative time. While, significant increase in CA15-3 was observed from both the pre- and post-operative time to after one year of follow up (P=0.006). Thus, serum CA15-3 concentration could be used on evaluating and monitoring the treatment of BC cases, among both metastatic and non-metastatic BC cases. This finding also confirmed the beneficial role of serum CA15-3 concentration on monitoring patient's response and predicting outcome. In agreement with the current study previous literature stated that the increase in CA15-3 may proceed clinically for radiographic detection of recurrence with a mean lead time of six to nine months [(17)]**.** Thus, increased CA15-3 levels indicate a poor response to chemotherapy. Furthermore, **Ali *et al.*** [(4)] discovered higher CA15-3 levels in individuals with recurrence after three rounds of treatment. Furthermore, **Nam *et al.*** [(18)] reported that the amount of CA15-3 in metastatic BC patients might predict therapy response and prognosis. This tumor marker is more prevalent in advanced or MBCs than in early-stage BC [(2)]. Persistently increased tumor markers are linked to recurrence or treatment resistance, which might aid in evaluating therapy efficacy [(19)]. Unfortunately, it is rarely employed for screening because of its limited sensitivity and specificity for BC [(20)]. Nevertheless, another research indicated that the post-operative level had little predictive value for BC recurrence [(21)].

However, CA15-3 didn't show significant relations with the clinic-pathological features among the studied BC cases. **Ali *et al.*** [(4)] showed no link with age, menopausal status, or lymph node status, but they did discover a positive correlation between tumor size and the CA15-3 level of disease stages, which is in contrast to the current data. According to a different research, advanced BC patients with big tumors, LN metastases, or higher histological grades had greater preoperative blood CA15-3 levels. This implies that a higher tumor burden is correlated with an enhanced tumor marker [(22)]. Moreover, CA15-3 was linked to

aggressive characteristics such as advanced stage and higher grades, according to **Nam *et al.*** [18]. Furthermore, **Zhao *et al.*** [23] recently investigated the association between 961 BC patients' CA15-3 and the most crucial clinico-pathological traits. Higher incidences of increased CA15-3 levels were found in patients with larger tumor sizes ($p < 0:0001$) and nodal metastases ($p = 0:0001$).

Similarly, research by **Hameed *et al.*** [24] showed a strong correlation between a greater tumor size and lymph node metastasis with a higher preoperative CA15-3 level. This suggests that greater CA15-3 has predictive value since it is connected with an increased tumor burden. The fact that we looked at BC patients in their early stages and that CA15-3 level is known to be correlated with tumor size, clinical stage, receptor expression, metastasis, and recurrence may have contributed to this discrepancy [16].

In addition, we studied whether CA15-3 level could be used as a surveillance marker to evaluate the efficiency of chemotherapy and to predict the disease free survival (DFS) among the studied sample, and the results indicated that CA15-3 didn't affect the DFS among the studied BC cases. On the other hand, according to many studies, individuals with higher CA15-3 tumor marker levels had worse OS and DFS than those whose levels were within the normal range [18, 25].

Thus, further larger, preferred multicenter, studies are needed to confirm the role of serum CA15-3 concentration on monitoring BC patient's outcome.

CONCLUSION

Our study showed that the studied biomarker is fairly simple, easy, cheap and little invasive methods, and could be used to determine disease response after treatment. Also, it was not necessary to mutually examine the pre- and post-operative serum level of CA15-3 to evaluate its role as this will not add a much more clinical benefit, and we can only examine the pre-operative serum CA15-3 concentration to avoid wasting time, effort and money.

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