# Elevated CEA and CA15-3 Serum Levels in Different Molecular Subtypes of Breast Cancer Have Prognostic Significance

Hajir Ali Al Saihati, PhD*

## ABSTRACT

**Breast cancer is the most frequent disease in women and the main cause of death from cancer, accounting for 23% of all cancer diagnoses and 14% of cancer deaths worldwide. Molecular indicators including hormone receptor status and human epidermal growth factor receptor 2 (HER2) expression are employed in addition to classic pathological measures such tumor size, tumor grade, and lymph node status. Many cancers employ serum tumor markers for screening, early detection of recurrence, and therapy. We enlisted the help of 36 women who had been diagnosed with stage I, stage III, invasive breast cancer that had been proven histologically and radiologically, and who had no clinical or radiological indications of metastases. Patients had surgery, either a modified radical mastectomy or a conservative mastectomy, and their preoperative CEA and CA15-3 levels, as well as regular follow-up, were all evaluated. Before surgery, we compared tumor marker levels, TNM staging, and molecular subtypes, as well as comparing tumor marker levels, TNM staging, and molecular subtypes. We discovered a relationship between preoperative CEA and CA15-3 serum levels and distinct molecular subtypes of breast cancer, as well as a link between preoperative CEA and CA15-3 serum levels and clinic-pathological tumor characteristics. TNM staging is one of the most important criterion. For distinct molecular subtypes of breast cancer, preoperative blood levels of tumor markers (CEA & CA15-3) have independent predictive significance.**

**Keywords: Carcinoembryonic antigen, Cancer antigen 15-3, Breast cancer**

## INTRODUCTION

Breast cancer is the most frequent disease in women and the leading cause of cancer mortality worldwide, accounting for 23% of all cancer diagnoses and 14% of cancer fatalities[1].

Breast cancer is the most prevalent and leading cause of cancer mortality among Egyptian women, accounting for 37.7% of all cancer deaths and 29.1% of all cancer deaths, respectively. A number of regional Egyptian cancer registries back up these findings[2].

In Arab nations, breast cancer is the most frequent malignancy, especially among young women who are diagnosed. The most common and acceptable treatment for locally advanced cancer is a complete mastectomy followed by adjuvant chemotherapy and radiation[3].

Serum tumor indicators are used for screening, early identification of recurrence, and treatment in many malignancies. In addition to traditional pathological parameters such as tumor size, tumor grade, and lymph node status, molecular markers such as hormone receptor status and human epidermal growth factor receptor 2 (HER2) expression are utilized[4].

Despite the fact that breast cancer is becoming more common, survival rates have improved in recent years as a result of extensive study into the disease's biology and behavior. Treatment failure, on the other hand, has a substantial impact on patients' quality of life and survival rate. To enhance prognosis, it is critical to develop accurate prognostic markers to aid decision-making throughout breast cancer treatment[5].

The following are the four molecular subtypes of breast cancer: The four forms of luminal tumors are: luminal A (ER+ and/or PR+, HER2-, Ki-6714 percent); luminal B (ER+ and/or PR+, HER2+ and/or Ki-6714 percent); HER2 positive (ER- and PR-, HER2+); and triple-negative (ER- and PR-, HER2+) (ER- and PR-, HER2-). For more than 30 years, the most extensively utilized blood tumor markers in breast cancer research have been carcinoembryonic antigen (CEA) and cancer antigen 15–3 (CA15-3)[4].

The goal of this study is to discover if preoperative serum CEA and CA15-3 levels in breast cancer patients have any predictive value and if they are linked to clinicopathological characteristics.

## PATIENTS

The researchers looked at 36 women who had breast cancer and had either a conservative or a modified radical mastectomy.

### Inclusion Criteria:

- A female between the ages of 18 and 75 is the patient.
- Invasive breast cancer stages I-III
- The patient desired a mastectomy or a breast-conserving procedure.
- The patient's signed consent; adjuvant chemotherapy, adjuvant radiation, and adjuvant endocrine treatment were all performed in accordance with international norms.

### Exclusion Criteria:

- Breast cancer that has advanced to the fourth stage of the disease.
- Carcinoma in situ is a malignancy that develops inside the body.
- Cases of neoadjuvant chemotherapy.
- Patient who has had a partial surgical removal or who has a little margin of error.

### Preoperative evaluation:

- A medical history, a comprehensive physical examination, and cardiologic consultation are all part of the clinical evaluation.
- A complete blood count, liver function tests, and kidney function

* Assistant Professor
Department of Clinical Laboratory Sciences
Faculty of Applied Medical Sciences, University of Hafr Al Batin
Email: hajirsh@uhb.edu.sa

tests are all part of the laboratory assessment.
- A chest X-ray and a pelvic and abdominal ultrasound.

### Treatment

Following a preoperative evaluation, surgery was performed, which comprised a conservative or modified radical mastectomy, as well as appropriate adjuvant chemotherapy, adjuvant radiation, and endocrine treatment, all in accordance with international guidelines. Prior to surgery, CEA and CA15-3 levels were measured, as well as at regular intervals after surgery and adjuvant therapy. An automated electrochemistry luminescence immunoassay system was used to assess the levels of CEA and CA15-3 in serum (ROCHE E170; Roche, Germany). The cut-off values for CEA and CA15-3 were 5.0 ng/mL and 25U/mL, respectively, and the result was classed as positive or negative for the marker if the level was above or below the cut-off value, and the pattern of these tumour measures was compared to various prognostic indicators.

### Prognostic parameters:

- Age: There are two age groups: those under 35 and those beyond 35.
- The TNM staging.
- The tumor's size and location.
- Lymph node metastasis

**Histologic grading:** It was completed using Bloom and Richardson's criteria. The presence of tumour emboli in peritumoral lymphatic spaces, capillaries, or post capillary venules was characterized as lymphatic vascular invasion.
**ER status and PR status:** If more than 10% of the tumor cells were stained, it was declared positive.
**HER2-positivity:** The findings were confirmed using a fluorescence in situ hybridization (FISH) assay for HER2. A fluorescence in situ hybridization (FISH) test for HER2 and a 2+ or 3+ score from an immunohistochemical examination were used to corroborate the findings.
**ki-67:** In a certain microscopic field, the ratio of positive cells to total cells was evaluated; "negative" was defined as less than 10% positivity, "weakly positive" as 10-25 percent positivity, "positive" as 26-50 percent positivity, and "strongly positive" as more than 50 percent positivity.

## Statistical Analysis

SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA) and MedCalc for Windows version 13 (SPSS Inc., Chicago, IL, USA) were used to analyse all data (MedCalc Software bvba, Ostend, Belgium).
The quantitative data were given a mean, SD, median (interquartile range), and range (minimum – maximum) whereas the qualitative data were given a number (percentage).

The Shapiro-Wilk test was used to ensure that continuous variables were normal. The Mann-Whitney U test was used to compare two groups of regularly distributed data, whereas the independent Student t-test was used to compare two groups of non-normally distributed data.

The Kruskal-Wallis H test was used to analyze non-normally distributed data, whereas the one-way ANOVA was used to analyze more than two sets of regularly distributed data. Friedman's test was used to evaluate non-normally distributed data, while repeated measure ANOVA was used to evaluate normally distributed data with more than two dependent groups. In repeated head-to-head comparisons, the Paired t test for normally distributed data and the Wilcoxon signed ranks test for non-normally distributed data were employed as posthoc testing. The Chi-square (2) test was used to compare percent of categorical variables.

All of the tests were conducted in pairs. P 0.05 was considered statistically significant, p 0.01 was considered very statistically significant (HS), and p 0.05 was considered non-statistically significant (NS).

## RESULTS

There were 36 patients in all, with 77.8% of them being over 35 years old and 88.9% of them having invasive duct carcinoma verified by histology. Grade II tumors were found in 31 (86.1%) of the patients, with 47.2 percent having pathological T2 and 36.1 percent having pathological N2. In the third stage of their sickness, 50% of the patients were (table 1).

There were 21 people with +ve ER receptors, the same as those with +ve PR receptors (58.8%), and 33.3 percent had +ve her2 receptors. The majority of patients had high Ki67 values (61.1 percent). In terms of molecular subtypes, luminal A tumors were found in 15 (41.7%) of the patients, whereas HER-2 amplification was seen in 6 (16.7%). (table 2).

Only 15 of the 36 patients who got chemotherapy and radiation received hormone therapy, with 12 of them getting Trastuzumab (table 3).

CEA and CA15-3 levels in the blood were found to be elevated in 5 (13.9%) and 6 (16.7%) of the subjects, respectively (table 4).

CEA levels were substantially higher in patients with Luminal B and HER2 positive tumors (p=0.03) than in patients with Luminal A and triple-negative tumours (p=0.03) (table 5).

CA15 3 levels were substantially higher in patients with HER2 positive and triple-negative tumors (p=0.02) than in patients with Luminal A and Luminal B malignancies (p=0.02) (table 6).

Higher CEA and CA15-3 readings were associated with larger primary tumors (p=0.001), axillary lymph node status (p=0.001), and TNM stage (p=0.03 for CEA and p=0.04 for CA15-3). Age, pathological type, tumor grade, serum ki67 level, hormone receptor status, or her2-neu status were not linked to increased CEA and CA15-3 levels (table 7).

Figure 1 illustrates the trend of serum tumor marker readings after 3, 6, 9, and 12 months compared to baseline values; tumor marker levels drop significantly following surgical excision of the tumor, and the fall continues after various treatment modalities (chemotherapy, radiotherapy, hormonal therapy and target therapy). No patient developed local or regional recurrence or distant spread over the study's duration.

**Table 1:** Patient's characteristics

| Variable | No | |
|---|---|---|
| | NO % | |
| **Age group:** | | |
| < 35 years | 8 | 22.2 |
| >35 years. | 28 | 77.8 |
| **Pathology:** | | |
| IDS | 32 | 88.9 |
| ILC | 4 | 11.1 |

| | | |
|---|---|---|
| **Grade:** | | |
| Grade 1 | 1 | 2.8 |
| Grade II | 31 | 86.1 |
| Grade HI | 4 | 11.1 |
| **T:** | | |
| T1 | 2 | 5.6 |
| T2 | 17 | 47.2 |
| T3 | 13 | 36.1 |
| T4 | 4 | 11.1 |
| **N:** | | |
| NO | 9 | 2.5 |
| N1 | 10 | 27.8 |
| N2 | 13 | 36.1 |
| N3 | 4 | 11.1 |
| **Stage:** | | |
| Stage I | 4 | 11.1 |
| Stage II | 14 | 38. 9 |
| Stage III | 18 | 50 |

**Table 2:** Molecular subtypes

| Variable | (N=36) | |
|---|---|---|
| | NO | % |
| **ER:** | | |
| -ye | 15 | 41.7 |
| +ve | 21 | 58.3 |
| **PR:** | | |
| -ye | 15 | 41.7 |
| +ve | 21 | 58.3 |
| **Her 2:** | | |
| -ye | 24 | 66.7 |
| +ve | 12 | 33.3 |
| **Ki27:** | | |
| Low | 14 | 38.9 |
| High | 22 | 61.1 |
| **Subtype:** | | |
| Lumina/ A | 15 | 41.7 |
| Lumina/ B | 8 | 22.2 |
| HER2ainpVied | 6 | 16.7 |
| TNBC | 7 | 19.4 |

**Table 3:** Adjuvant therapy

| Variable | (N=36) | |
|---|---|---|
| | NO | % |
| **Chemotherapy**: | | |
| No | 4 | 11.1 |
| Yes | 32 | 88.9 |
| **Radiotherapy:** | | |
| No | 4 | 11.1 |
| Yes | 32 | 88.9 |
| **Hormonal:** | | |
| Na | 15 | 41.7 |
| Yes | 21 | 58.3 |
| **Trastuzumab**: | | |
| Na | 24 | 66.7 |
| Yes | 12 | 33.3 |

**Table 4:** Preoperative tumor markers level (CEA and CA15-3)

| Pre-Operation Level | Normal Nr70 | Elevated N(%) | Mean ± SD Median (Range) |
|---|---|---|---|
| CEA | 31 (86.1%) | 5 (13.9%) | |
| CA15-3 | 30(83.3%) | 6 (16.7%) | |

**Table 5:** Correlation between pre-operative CEA and molecular subtypes

| | Molecular subtypes | N | CEA Mean | SD | Range | K | P |
|---|---|---|---|---|---|---|---|
| Pre-operation | LAuninal A | 15 | 8.09 | 13.76 | 1 4 64.4 | 7.14 | OAP* |
| | Luminal B | 8 | 38.57* | 61.47 | 1.2 132.0 | | |
| | Her 2 amplified | 6 | 8.48* | 20.23 | 1.2 66.0 | | |
| | TNBC | 7 | 6.65 | 16.64 | 1.4 64.4 | | |

**Table 6:** Correlation between pre-operative CA15-3 and molecular subtypes

| Time | Subtype | N | CA 15-3 Mean | SD | Range | K | P |
|---|---|---|---|---|---|---|---|
| Pre-operation | LAuninal A | 15 | 25.65 | 25.25 | 11.8-70.6 | 8.84 | 0.02 |
| | Luminal B | 8 | 26.56 | 29.28 | 11.0-127.5 | | |
| | Her 2 amplified | 6 | 32.09* | 36.35 | 12.9-136.2 | | |
| | TNBC | 7 | 28.1* | 17.49 | 15.8-65.8 | | |

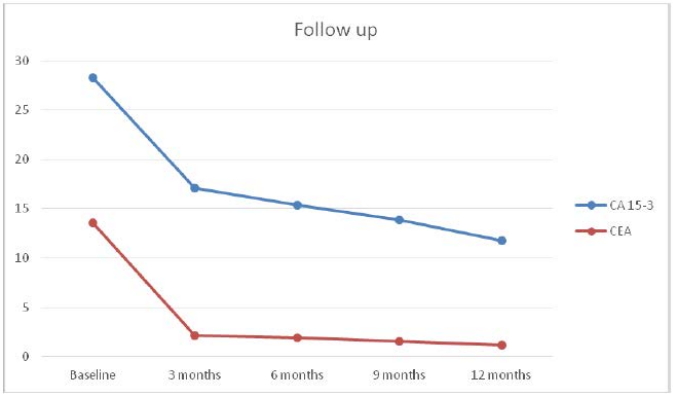


**Figure 1:** Pattern of serum tumor markers measurements at 3,6,9,12 months in relation to baseline values

## DISCUSSION

Breast cancer is the most prevalent and leading cause of cancer mortality among Egyptian women, accounting for 37.7% of all cancer deaths and 29.1% of all cancer deaths, respectively. A number of regional Egyptian cancer registries back up these findings[2].

Despite the fact that breast cancer is becoming more common, survival rates have improved in recent years as a result of extensive study into the disease's biology and behavior. Treatment failure, on the other hand, has a substantial impact on patients' quality of life and survival rate. To enhance prognosis, it is critical to develop accurate prognostic markers to aid decision-making throughout breast cancer treatment.

The goal of this study was to see how important higher blood levels of tumor markers like CEA and CA15-3 are in various molecular subtypes of breast cancer.

The participants in this research ranged from stage I to stage III invasive breast cancer. Preoperative CEA and CA15-3 levels were examined in 36 patients; high CEA levels were found in 5 (13.9%) patients, whereas elevated CA15-3 levels were seen in 6 patients (16.7 percent).

**Table 7:** Relation between pre-operative CEA and CA15-3 levels and general characteristics and clinical data of the studied group

| General charachteristices | | CEA | | | | P | CA 15-3 | | | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Normal (n=31) | | Elevated (n=5) | | | Normal (n=30) (n=6) | | Elevated (n=30) | | |
| | | NO | % | NO | % | | NO | % | NO | % | |
| Age group | < 35 years | 6 | 19.4 | 2 | 40 | 0.31 | 8 | 26.7 | 0 | 0 | 0.30 |
| | >35 years | 2.5 | 80.6 | 3 | 60 | NS | 22 | 73.3 | 6 | 100 | NS |
| Pathology | IDS | *2.7* | 87.1 | 5 | 100 | 0.63 | 26 | 86.6 | 6 | 100 | 0.56 |
| | ILA | 4 | 12.9 | 0 | 0 | NS | 4 | 13.3 | 0 | 0 | NS |
| Grade | GNadel | 1 | 3.2 | 0 | 0 | 0.74 NS | 1 | 3.3 | 0 | 0 | 0.56 |
| | Gracie I | 27 | 87.1 | 4 | 80 | | 25 | 83.3 | 6 | 100 | NS |
| | Gracie III | 3 | 9.7 | 1 | 20 | | 4 | 13.3 | 0 | 0 | |
| T: | 1 | 2 | 6.5 | 0 | 0 | | 2 | 6.7 | 0 | 0 | |
| | 2 | 16 | 51.6 | 1 | 20 | <0.001 | 15 | 50 | 2 | 33.3 | <0.001 |
| | 3 | 13 | 41.9 | 0 | 0 | -- | 13 | 43.3 | 0 | 0 | |
| | 4 | 0 | 0 | 4 | 80 | | 0 | 0 | 4 | 66.7 | |
| N: | 0 | 9 | 29 | 0 | 0 | | 8 | 26.7 | 1 | 16.7 | |
| | 1 | 9 | 29 | 1 | 20 | <0.001 | 9 | 30 | 1 | 16.7 | <0.001 |
| | 2 | 13 | 41.9 | 0 | 40 | -- | 13 | 43.3 | 0 | 0 | |
| | 3 | 0 | 0 | 4 | 40 | | 0 | 0 | 4 | 66.7 | |
| Stage | Stage 1 | 4 | 12.9 | 0 | 0 | | 4 | 13.3 | 0 | 0 | <0.04* |
| | Stage II | 13 | 41.9 | 1 | 2.0 | <.0.03* | 12 | 40 | 2 | 33.3 | |
| | Stage III | 14 | 45.2 | 4 | 80 | | 14 | 46.7 | 4 | 66.7 | |
| ER : | -ye | 13 | 41.9 | 2 | 40 | 1 | 12 | 40 | 3 | 50 | 0.68 |
| | +ve | 18 | 58.1 | 3 | 60 | NS | 18 | 60 | 3 | 50 | NS |
| PR: | -ye | 13 | 41.9 | 2 | 40 | 1 | 12 | 40 | 3 | 50 | 0.68 |
| | -1.e | 18 | 58.1 | 3 | 60 | NS | 18 | 60 | 3 | 50 | NS |
| Her2 : | -ve | 22 | 71 | 2 | 40 | 0.31 | 21 | 70 | 3 | 50 | 0.38 |
| | -ve | 9 | 29 | 3 | 60 | NS | 9 | 30 | 3 | 50 | NS |
| Ki27 | Low | 13 | 41.9 | 1 | 20 | 0.63 | 13 | 43.3 | 1 | 16.7 | 0.37 |
| | High | 18 | 58.1 | 4 | 80 | NS | 17 | 56.7 | 5 | 83.3 | NS |

This is in line with Park et al.[6] who observed that 12.4 percent of patients had high CA15-3 levels and 10.7% had elevated CEA, and Shao et al. [(7)], who discovered that 13.9 percent of patients had elevated CA15-3 levels and 10.9 percent had elevated CEA.

According to Park et al.[6], TNM staging revealed that stage I (38.4%), stage II (44.4%), and stage III (17.2%) were present in half of the patients (50%) and the other half (50%) respectively. However, our analysis revealed that stage III was present in half of the patients (50%) and the other half (50%) respectively (11.1 percent stage I and 38.9 percent stage II).

We identified a statistically significant link between TNM staging and heightened levels of tumor markers in our research; the higher the preoperative serum marker levels, the more advanced the tumor stage.

TNM staging indicated that stage I (38.4%), stage II (44.4%), and stage III (17.2%) were present in half of the patients (50%) and the other half (50%) of the patients, respectively, according to Park et al.[6]. Our research found that stage III was present in half of the patients (50%) and the other half (50%) of the patients, respectively (11.1 percent stage I and 38.9 percent stage II).

In our research, we discovered a statistically significant correlation between TNM staging and elevated levels of tumor markers; the greater preoperative serum marker levels, the more advanced the tumor stage. The levels of CEA and CA15-3 are linked to tumor burden factors such tumor size and lymph node status. The increased tumor marker values in N3 were statistically significant (40 percent for CEA and 66.1 percent for CA15-3, respectively). In comparison, Shao et al[7] and Lee et al[8].

The luminal A subtype was the most frequent, accounting for 41.7 percent of all molecular subtypes, followed by luminal B (22.2 percent), Her 2 neu +ve (16.7 percent), and TNBC (16.7 percent) (19.4 percent). This is in line with the findings of Gong et al.[9], who found luminal A to be 62.9 percent, luminal B to be 15.3 percent, HER-2 +ve to be 15.5 percent, and TNBC to be 13.4%.

Preoperative CEA levels were considerably higher in patients with HER2 positive tumors (p =0.03) but not with ER expression in the San-gang et al.[10] research, but preoperative CA15-3 levels were significantly higher in ER negative patients (p =0.02). CEA values were considerably greater in patients with HER2 positive tumors in the Shao et al.[7] investigations.

The luminal A subtype was the most common, accounting for 41.7 percent of all molecular subtypes, with luminal B (22.2 percent), Her 2 neu +ve (16.7 percent), and TNBC (16.7 percent) following closely behind (19.4 percent). Gong et al.[9] discovered luminal A to be 62.9 percent, luminal B to be 15.3 percent, HER-2 +ve to be 15.5 percent, and TNBC to be 13.4 percent.

In the San-gang et al.[10] study, preoperative CEA levels were substantially higher in patients with HER2 positive tumors (p =0.03) but not with ER expression, although preoperative CA15-3 levels were significantly higher in ER negative patients (p =0.02). In the Shao et al.[7] study, CEA levels were significantly higher in patients with HER2 positive tumors. This study demonstrated no relationship between histological grading and preoperative tumor marker levels, which agrees with San-gang et al's findings[10].

In 41.7 percent of patients, progesterone receptors (PR) were discovered to be negative, whereas in 58.3 percent, they were found to be positive. PR was found to be positive in 45.6 percent of the people in the Park et al.[6] investigations.

Unlike Lee et al.[8], we discovered no statistically significant connection between PR and preoperative tumor marker levels in our investigation.

A low ki67 level was found in 38.9% of patients in our research, whereas a high level was found in 61.1 percent. Shao et al.[7] observed that 14.8 percent of patients had increased ki67 levels in their blood, with high levels found in the majority of cases (82.2 percent).

According to San-gang et al., there was no statistically significant association between ki67 and tumor marker levels[10].

## CONCLUSION

**For distinct molecular subtypes of breast cancer, preoperative blood levels of tumor markers (CEA and CA15-3) have independent prognostic value.**

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**Competing Interest:** None.

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