

Evaluation of CA 15-3, Her-2/neu and estrogen/progesterone status in breast cancer patients treated by surgical removal and chemotherapy

Taha H Taha Al-siagh*, Shahbaa A Al-bayati**, Faris A Ahmed**

Departments of *surgery, **Physiology, Ninevah college of Medicine, University of Mosul

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ABSTRACT

Objective: This study was conducted to evaluate serum CA 15-3 in breast cancer patients after surgery and chemotherapy. The relationship between serum CA 15-3 and Her-2/neu, estrogen, or progesterone receptors were also studied in the breast cancer patients.

Patients and methods: This study was conducted at Al-Jammhori Teaching Hospital, and Al-Zahrawi Private Hospital, Mosul, Iraq, during the period from January to Jun 2011. Fifty breast cancer women diagnosed by using triple assessments by a surgeon (history examination, ultrasound mammography, and fine needle aspiration cytology) were included in the study. Blood samples (5 mL) were taken from the patients and analyzed for serum CA 15-3 by monoclonal antibody technique. The blood samples were taken from the patients a week before and after one week of the operation and other blood samples were taken after two weeks of the first cycle of chemotherapy treatment. Combination therapy of 5-fluorouracil, epirubicin, cyclophosphamide, for three cycles every 21 days, was given to the patients, followed by three cycles docytaxil every 21 days. Samples from the tumor on paraffin sections taken from the patients were analyzed for Her-2/neu, estrogen and progesterone receptors by immunochemical methods.

Results: Serum CA 15-3 was significantly decreased by 54% ($p \leq 0.001$) after surgical removal of the cancer compared with results before surgery. Chemotherapy of the patients further decreased serum CA 15-3 significantly by 78% ($p \leq 0.001$) compared with the results before surgery. CA 15-3 and tumor size in positive Her-2/neu patients were significantly higher than in negative Her-2/neu patients. However, serum CA 15-3 and tumor size in positive estrogen patients were significantly lower than in negative estrogen patients. In progesterone positive patient's serum CA 15-3 was only significantly ($p \leq 0.001$) lower than in the negative progesterone patients. Mean ages was significantly ($p \leq 0.001$) higher in positive Her-2/neu patients than in negative Her-2/neu patients. However, mean ages was significantly ($p \leq 0.001$) higher in positive estrogen and progesterone patients than in negative receptors. Metastasis occurred in 39 patients (78 %) out of the studied patients. Negative significant correlation was noticed between Her 2/neu and estrogen receptors ($r = -0.745$, $p \leq 0.001$). Negative significant correlation was also noticed between Her 2/neu and progesterone receptors ($r = -0.786$, $p \leq 0.001$). Weak but significant correlation was found between serum CA 15-3 and tumor size ($r = 0.281$, $p \leq 0.05$).

Conclusion: CA 15-3 and Her 2/neu are useful tumor markers for diagnosis of breast cancer in the treatment through surgery and chemotherapy. The co-expression between hormonal and Her-2/neu receptors is negative. The need for more sensitive tumor markers is still wanted.

الخلاصة

الهدف: تهدف هذه الدراسة تقييم مصل دم CA 15-3 في المرضى المصابين بسرطان الثدي بعد الجراحة وبعد العلاج الدوائي. كذلك تم دراسة العلاقة بين مصل دم CA 15-3 و مستقبلات Her-2/neu والاستروجين والبروجستيرون.

المرضى وطرائق العمل: تم اجراء هذه الدراسة في المستشفى الجمهوري التعليمي والمستشفى الزهراوي الاهلي في الموصل، العراق، خلال الفترة من كانون الثاني الى حزيران 2011. شملت الدراسة خمسين امرأة مصابة بسرطان الثدي شخصت من قبل جراح مختص باستعمال ثلاثة وسائل (تاريخ المرض، تخطيط الثدي بالأموح فوت الصوتية والسحب الخلوي بواسطة الإبرة الرفيعة). تم سحب عينات الدم (5 ملم) من المرضى وتم تحليلها لمصل دم CA 15-3 باستعمال تقنية المضادات المناعية الأحادية. اخذت عينات الدم من المرضى بأسبوع قبل وبعد إجراء العملية الجراحية وعينة اخرى بعد أسبوعين من أول جرعة علاجية. شمل العلاج كل من 5- fluorouracil, epirubicin, cyclophosphamide متحدا كل 21 يوما ولثلاث دورات. متبوعا بثلاث دورات من عقار docytaxil كل 21 يوما بين دورة وأخرى. تم تحليل عينات السرطان في مقاطع البارافين من المرضى لكل من مستقبلات Her-2/neu والاستروجين والبروجستيرون بالطريقة الكيميائية المناعية.

النتائج: كان مصل دم CA 15-3 اقل معنويا بمستوى دلالة 0.001 (54%) بعد عملية ازالة السرطان مقارنة بتلك النتائج قبل العملية. وقلل العلاج السرطاني للمرضى مصل دم CA 15-3 معنويا ب مستوى دلالة 0.001 (78%) مقارنة بالنتائج قبل الجراحة. وكان مصل دم CA 15-3 وحجم السرطان في المرضى الموجب مستقبلات Her-2/neu اعلى معنويا بمستوى دلالة 0.001 منه في المرضى السالب مستقبلاتهم. ومن ناحية اخرى كان مصل دم CA 15-3 وحجم السرطان في المرضى الموجب مستقبلات الاستروجين اقل معنويا بمستوى دلالة 0.001 في المرضى السالبة مستقبلاتهم. اما في المرضى الموجبة مستقبلات البروجستيرون كان مصل دم CA 15-3 فقط اقل معنويا بمستوى دلالة 0.001 منه في المرضى السالبة مستقبلاتهم. وكان معدل العمر اعلى معنويا بمستوى دلالة 0.001 في المرضى الموجبة مستقبلات Her 2/neu منه في المرضى السالبة مستقبلاتهم. ومن ناحية اخرى كان معدل العمر للمرضى اعلى معنويا بمستوى دلالة 0.001 في المرضى الموجبة مستقبلاتهم لكل من الاستروجين والبروجستيرون مقارنة بالمرضى السالبة مستقبلاتهم. وحدث انتشار السرطان في 39 مريضا (78%) من كل المرضى في الدراسة. وكان هناك علاقة سلبية معنوية بين مستقبلات Her 2/neu ومستقبلات الاستروجين بمستوى دلالة $R=0.754, p < 0.001$ كذلك كان هناك علاقة سلبية معنوية بين مستقبلات Her 2/neu ومستقبلات البروجستيرون $r = -0.786, p \leq 0.001$ وحجم السرطان بمستوى دلالة 0.05. $r=0.281$.

الاستنتاج: ان مصل دم CA 15-3 ومستقبلات Her 2/neu هو مفيد في تشخيص سرطان الثدي عند العلاج عن طريق الجراحة والعلاج الدوائي. وان العلاقة بين مستقبلات الهرمونات ومستقبلات Her 2/neu سلبية. وتبقى الحاجة إلى اكتشاف معلمات انتقائية لسرطان الثدي.

Number of tumor associated antigens has been evaluated for monitoring or detecting breast cancer. CA 15-3 is the product of Mucin-1 gene, and mucin are aberrantly over expressed in many adenocarcinoma in an underglycosylated form and then shed into the circulation¹. CA 15-3

appears to be useful and more effective².

There have been reports showing worse prognosis in patients with high concentration of CA 15-3³. CA 15-3 has been shown to be an independent predictor of first recurrence as well as a powerful prognostic indicator in

patients with advanced breast cancer⁴. Although its usefulness remains uncertain and American Society of Clinical Oncology guideline do not recommend its use for follow up⁵.

Her-2/neu oncogen has been extensively investigated as a prognostic factor as recently as a predictor of response to chemotherapy or endocrine therapy⁶. Over expression/amplication of the Her-2/neu has been associated with worse outcome in patients with breast cancer⁷.

Tumor that express both estrogen and progesterone receptor have the greatest benefit from hormonal therapy and help physicians for therapy⁸. Negative estrogen and progesterone tumors tend to be of high grade and have worse prognosis^{9,10}. There is negative correlation between hormone receptor expression and Her-2/neu amplification, but not all high grade breast cancer showed Her-2/neu positive status¹¹.

This study was conducted to evaluate serum CA 15-3 in breast cancer patients after surgery and chemotherapy. The relationship between serum CA 15-3 and Her-2/neu, estrogen, or progesterone receptors were also studied.

Patients and methods

This study was conducted at Al-Jammhori Teaching Hospital, and Al-Zahrawi Private Hospital, Mosul, Iraq, during the period from January to Jun 2011. Fifty breast cancer women diagnosed by using triple assessments by a surgeon (history examination, ultrasound mammography, and fine

needle aspiration cytology) were included in this study. The patient ages ranged between 22 to 85 years (mean \pm s.d: 48.3 ± 14.7 years).

Blood samples (5 mL) were taken from the patients and analysed for serum CA 15-3 by monoclonal antibody technique (Minividas, USA, kids by Biomerieux company, France). The blood samples were taken from the patients a week before operation and also after one week of the operation. Other blood samples were taken after two weeks of the first cycle of chemotherapy treatment. Combination therapy of 5-fluorouracil 500 mg/m^2 , epirubicin 100 mg/m^2 , and cyclophosphamide 500 mg/m^2 , for three cycles every 21 days, was given to the patients, followed by three cycles docytaxil 100 mg/m^2 every 21 days. Patients in immune histochemical staining for Her-2/neu positive were given trazotuzumab 6 mg/Kg BW with docytaxil every 21 days for one year started after 21 days of the last dose of docytaxil cycle. Samples from the tumor on paraffin sections taken from the patients were sent to Histopathology Department in Al-Jammhori Teaching Hospital and analysed for Her 2, estrogen and progesterone receptors by immunochemical method (Dako company, Danemark).

Data are presented as mean \pm s.d. and were analyzed by using paired t-test to compare the follow up parameters. Q squire test was used to compare non-parametric measurements. SPSS package version 17 was used for analysis.

Results

Table 1. Serum CA 15-3 in breast cancer patients under surgical and chemotherapy treatment (n= 50)

Patients	CA 15-3 (U/mL)
Before surgery	84.42± 19.08
After surgery	38.88± 13.77 ^a
sAfter chemotherapy	18.62± 12.29 ^b

^a $p \leq 0.001$ vs before surgery, ^b $p \leq 0.001$ vs before surgery

Table 1 shows that serum CA 15-3 was significantly decreased ($p \leq 0.001$) after surgical removal of the cancer compared with results before surgery. Chemotherapy of the patients further decreased serum CA 15-3 significantly ($p \leq 0.001$) compared with the results before surgery.

Table 2 shows that CA 15-3 and tumor size in positive Her-2/neu patients were significantly higher than in negative Her-2/neu patients. However, serum CA 15-3 and tumor size in positive estrogen patients were significantly lower than in negative estrogen patients. In progesterone positive patient's serum CA 15-3 was only significantly ($p \leq 0.001$) lower than in the negative progesterone patients. Mean ages was significantly ($p \leq 0.001$) higher in positive Her-2/neu patients than in negative Her-2/neu patients.

However, mean ages was significantly ($p \leq 0.001$) higher in positive estrogen and progesterone patients than in negative receptors.

Metastasis was found in 39 patients (78%) out of the studied patients (node, lung, liver, or bone). Thirty four out of 50 of the patients were married and the number of children for these patients was between 0 and 7 children.

Negative significant correlation was noticed between Her-2/neu and estrogen receptors ($r = -0.745$, $p \leq 0.001$). Negative significant correlation was also noticed between Her-2/neu and progesterone receptors ($r = -0.786$, $p \leq 0.001$). Weak but significant correlation was found between serum CA 15-3 and tumor size ($r = 0.281$, $p \leq 0.05$)

Table 2. Serum CA 15-3 in breast cancer patients with positive and negative Her 2/neu, estrogen and progesterone receptors (n= 50 for all patients)

Receptors	n	CA 15-3 (U/mL)	Tumor size (cm)	Age (years)
Her 2/neu (positive)	19	100.47± 12.94	4.63± 2.41	40.8±9.8
Her 2/neu (negative)	31	74.03± 12.41**	2.58± 1.89*	53.1±15.1**
Estrogen (positive)	31	76.39± 16.39	2.74± 1.84	52.5±14.9
Estrogen (negative)	19	99.37± 14.44**	4.37± 2.67*	41.8±11.8**
Progesterone (positive)	32	76.97± 15.96	2.32± 2.32	52.2±15.3
Progesterone (negative)	18	99.56± 15.98**	4.11± 2.23	41.7±10**

* $p \leq 0.05$ vs values with positive receptors, ** $p \leq 0.001$ vs values with positive receptors

Discussion

In the present study, serum CA 15-3 was decreased significantly after surgical removal of the cancer, chemotherapy further significantly decreased serum CA 15-3. These results are consistent with other workers¹². High preoperative CA 15-3 and CEA levels may reflect tumor burden and are associated with advanced disease and poor outcome¹³. CA 15-3 is particularly valuable for treatment monitoring in patients that have disease that cannot be evaluated using existing radiological procedure¹⁴. However, pre-operative serum CA 15-3 measurement is of little value, especially in patients with early stage breast cancer and are not useful in the therapeutic decision-making of patients with breast cancer¹⁵. In addition, the time elapsed between CA 15-3 increase and clinically confirmed progression was very short for follow up study¹⁶.

In Her-2/neu positive patients, serum CA 15-3 was significantly higher than in Her-2/neu negative patients. Overexpression/amplification of the Her 2/neu has been associated with a worse outcome in patients with breast cancer⁷. CA 15-3 was associated with age, tumor size, nodal involvement, and Her 2 expression¹⁷. Serum Her 2 and CA 15-3 in breast cancer were useful markers for predicting aggressive behavior of tumor and elevated serum levels are associated with breast cancer relapse¹². The combination of the increased serum Her-2/new and increased serum CA 15-3 predicts a worse prognosis than increased CA 15 alone¹⁸.

Metastasis of cancer was high (78%) in the present patients. It is commonly accepted that the earlier the detection of the disease the better the prognosis. Estrogen, progesterone, and Her-2/neu receptors are independent prognostic factors for locoregional recurrence and survival in node positive breast cancer patients treated by mastectomy¹⁹. These biomarkers showed significant concordance between primary tumors and lymph node metastasis²⁰. Moreover, the survival analyses showed that the expression of biomarkers in lymph node metastasis can provide prognostic information when no analysis of primary tumor can be done²¹. CA 15-3 provided significant additional information to the common prognostic factors to predict the occurrence of metastasis¹⁷.

In the present study, serum CA 15-3 was significantly lower in the hormonal positive patients than in the negative hormonal patients. The positivity rates of serum CA 15-3 significantly correlated with estrogen and progesterone status²².

Negative significant correlation was noticed between the present Her-2/neu and estrogen or progesterone receptors. These results are consistent with other workers^{23,24}. However, the co-expression of hormonal receptors with Her-2/neu is infrequent²⁵. Her-2 was associated with bad prognosis and correlated with the grade of tumor^{6,26}. High grade tumors tend to be estrogen negative and Her-2 positive, estrogen negative tumors tend to be of high grade and worse prognosis tumors¹⁰.

In the present study, mean ages in the Her-2/neu positive was

significantly lower than that in negative receptors. However, ages were significantly higher in hormonal positive receptors than that in the negative receptors. No correlation was found between Her-2 receptor and ages of the breast cancer patients²⁴.

In conclusion, CA 15-3 and Her 2/neu are useful tumor markers for diagnosis of breast cancer through surgery and chemotherapy. The co-expression between hormonal and Her-2/neu receptors is negative. The need for more sensitive tumor markers is still wanted.

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