Original Article

Evaluation of Preoperative Predictive Values of Serum CA15-3 and CEA within Sudanese Women with Breast Cancer.

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Abstract

Objectives: Early detection of cancer comprises early diagnosis in symptomatic and screening of asymptomatic individuals. Our aim was to evaluate the significant values of carbohydrate antigen 15-3 (CA15-3) and/or Carcinoembryonic antigen (CEA) in women with breast cancer.

Design and setting: This case control study was conducted in Khartoum Teaching Hospital, Khartoum, Sudan.

Application of such measurement may be helpful within screening and early detection efforts in such a country like Sudan with poor resources.

Methods: We examined by serological radioimmuno-assay methods, significant elevation of CA15-3 and CEA serum samples obtained from 100 women of whom 40% and 35% were patients with histopathologically confirmed breast cancer and benign breast lumps respectively and the remaining 25% were apparently healthy controls.

Statistical analysis: Data were analyzed by using a computer SPSS program.

Results: Among the 75 patients with breast lumps, 33 (44%) and 31(37.3%) showed high CA15-3 and CEA levels respectively. Of the 40 carcinomas, high expressions of CA15-3 and CEA were found among 28(70%) and 24(60%) respectively. Notably, only 2(8%) of the controls showed lightly elevated CEA.

Conclusions: The obtained Specificity of 85.7%, 80% and sensitivity of 70%, 60% for CA15-3 and CEA correspondingly, support the combined application of both markers in screening for breast cancer.

Key words: Carcinoembryonic antigen, radioimmuno-assay, breast lump, histopathologically

Female breast cancer is by far the leading cancer in the Sudan[1]. The alarmingly high frequency of women presenting with advanced breast cancer to the Radiation Isotope Center Khartoum (RICK) and Gaziera Institute for Cancer treatment and Molecular Biology (GICMB), which are the only two oncology centers in the Sudan, has prompted looking for an investigation that might help in solving this real health problem. However, there were variations in the proportions of breast cancer, during the period 1990-2001, as shown in table1.

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The highest percentages were recorded in 1998 (38.4% of all female cancers), followed by the years, 2000, 1999 and 2001, which attended 36.03%, 35.2% and 32.4% respectively[1]. Furthermore, the records from GICMB, indicated the highest percentages (42%) of breast cancer[2].

CA 15-3 and CEA have been most widely used as serum tumor markers in follow-up and detection of breast cancer recurrence. In this study we have specifically focused upon the diagnostic implications and utility of preoperative CA 15-3 and CEA levels.

The incidence and mortality of breast cancer are high in the Sudan, and the frequency is increasing particularly amongst younger women[3]. This might not be entirely due to an increase in the at risk population, but may be due to a lower threshold and better recognition of new cases.

Table.1: Frequencies of breast cancer from 1990 – 2001 (RICK 2002).

Year	Breast Cancer	Percentage	Female Cancers
1990	200	21.3 %	941
1991	140	15.7 %	890
1992	327	26.1 %	1255
1993	162	11.1 %	1462
1994	255	27.3 %	934
1995	60	5.9 %	1018
1996	314	28.4 %	1106
1997	349	29 %	1203
1998	502	38.4 %	1306
1999	455	35.2 %	1293
2000	481	36.03 %	1335
2001	531	32.4 %	1639

Therefore, the assessment of the value of circulating tumor markers (CA15-3& CEA) for screening and preoperative predicting of breast cancer is very important.

High levels of CA15-3, during the post-therapeutic follow-up of a cancer of the breast indicated recurrence or metastasis in 75 to 90% of the cases[4-6]. A significant positive association was found between CA 15-3 concentrations and both tumor stage and the number of involved axillary lymph nodes[7].

Preoperative serum concentrations of CA15-3 appear to have a significant relation to outcome in patients with early breast carcinoma and may have a role in the rational selection of patients for appropriate adjuvant treatments[8].

A combination of CEA and CA15-3 has been reported to improve lead detection time in metastatic disease, more so than the use of either assay alone[9-12].

Materials and Methods

In this study we evaluated the role of serum CA15-3 and CEA as compared to histopathology in the investigation and screening for breast cancer within women with palpable breast lumps.

One hundred subjects were randomly selected for this study. Of whom 75 were patients with breast masses and 25 were apparently healthy controls. All studied subjects were females their ages ranging between 20- 75 years with a mean age of 42 years. Brief clinical and personal data were obtained from each patient during the interview and reviewing of the ethical consent.

Serum obtained from blood specimens was analyzed using radio- immuno assay for breast tumor markers using antiserum to CA-15-3 and CEA.

The CA15-3 or CEA assay is a two-step "sandwich" type assay in which two monoclonal mouse antibodies, directed against two different epitopes of the molecule, are employed.

Samples or standards were incubated in tubes coated with the monoclonal antibody, the contents of the tubes were then aspirated and the presence of CA15-3 or CEA in the sample was revealed by incubation with a second, ^{125}1-labeled monoclonal antibody. The contents of the tubes were aspirated after this second incubation and unbound labeled antibody was eliminated by washing.

The amount of bound reactivity measured in a gamma counter was proportional to the CA15-3 or CEA concentration.

CA15-3 or CEA assayed adopting the following steps: Number a duplicate series of anti-CA15-3, or CEA-coated tubes.
First incubation: a) Add 200 µl of standard or sample, control, prepared in to tubes prepared for the assay.b) Shake gently. c) Cover tubes.d) Incubate tubes for 2 hours at room temperature (18-25˚C) with continuous horizontal shaking (400rpm).
Washing: a. Aspirate carefully the contents of the tubes. b. Add 2 ml of distilled water to each and immediately aspirate carefully contents of the tubes. Wash once more. No liquid should remain in tubes.
Second incubation: a. Add 200 µl of tracer to tubes. b. Add 200 µl of tracer to 2 additional tubes to obtain total counts T.
c. Cover tubes.
d. Incubate tubes for 1 hour at room temperature (18-25˚C) with continuous horizontal shaking (400 rpm).
Washing:
a. Aspirate carefully the contents of the tubes (except of tubes T).
B. Rinse tubes (except tubes T) with 2 ml of distilled water and immediately aspirate carefully contents of the tubes. Wash once more. No trace of the dye should remain in the tubes.
Determine the radioactivity of the tubes in order to obtain bound (B) and total cpm (T).

Sample processing for histopathology

Biopsies of thickness of 10-mm in diameter were selected from tissues required for histopathology. These biopsies were fixed in neutral buffered formalin for 30 hours, and then processed in tissue processing machine using; Neutral buffered formalin for 30 minutes followed by ascending alcoholic concentrations70%, 90%, 3 changes of absolute ethanol one hour for each. The tissues were then placed in 2 changes of xylene 2 hours for each then transformed into 2 changes of molting paraffin wax for more than 4 hours.

Five sections of 5µm in thickness were obtained from formalin-fixed paraffin wax embedded tissues using a rotary microtome. Sections were stained using haematoxylin and eosin (Mayer's procedure)[13].

Results

A total of 75 women who presented to Khartoum Teaching Hospital with palpable breast masses, in addition to 25 apparently healthy subjects (controls) were studied. Their ages ranged from 20 to 75 years (mean=42).
As the histopathology is a gold standard, biopsies from the 75 patients with breast palpable lumps were investigated histopathologically. Breast cancer was detected among 40(53.3%). Within these 40 patients, high levels CA15-3 and CEA were determined in 28(70%) and 24(60%) respectively. Of the remaining 35(46.7%) patients with benign breast lumps, elevated levels of CA15-3 and CEA were detected among, 5(14.3%) and 7(20 %) correspondingly as shown in tables 2, 3.

Table 2: Description of CA15-3 versus histopathology results.

	CA15-3					
	Positive		Negative		Total	
Histopathology	N	%	N	%	N	%
Positive	28	37.3	12	16	40	53.3
Negative	5	6.7	30	40	35	46.7
Total	33	44	42	56	75	100

$P < 0.001$

Table 3. Description of CEA versus histopathology results according.

	CEA					
	Positive		Negative		Total	
Histopathology	N	%	N	%	N	%
Positive	24	32	16	21.3	40	53.3
Negative	7	9.3	28	37.4	35	46.7
Total	31	41.3	44	58.7	75	100

$P < 0.05$

Although, 12 (30%) of the patients that were histopathologically proved as having breast cancer were found with low (negative) levels of CA15-3, as well as 5 (14%) patients with those expected to reveal low levels (negative) have revealed higher levels, these findings showed a significant role for CA15-3 as a predictor for breast cancer ($P< 0.001$), as indicated in table.2.

Additionally, though 40% of patients with breast cancer were having low levels of CEA (negative) and 20% of those patients with benign breast lumps revealed elevated levels of CEA (positive), but also CEA have shown statistically significant important($P< 0.05$) as shown in table.3.

Thereafter, specificity of 85.7% and 80% as well as a sensitivity of 70% and 60%, as well as positive predictive values of 85% and 77.4% was credited for CA15-3 and CEA respectively. Additionally, negative predicative value 71.4% was determined for CA15-3, hence, 63.6% was obtained by CEA.

When comparing the results obtained by the two markers; they agreed in 21(28%) positive and 32(42.7%) negative results. Nonetheless, 10(13.3%) were detected positive by CEA and negative by CA15-3, on the other side, 12 (16%) were Negative by CEA and turned to be positive by CA15-3.

Concerning the 25 apparently healthy controls, only 2 subjects among the controls showed slightly elevated CEA levels.

The description of both markers findings versus histopathology is shown in table.4

Table 4. Description of CA15-3 and CEA findings versus histopathology results.

	CA15-3 and CEA					
	Positive		Negative		Total	
Histopathlogy	N	%	N	%	N	%
Positive	31	77.5	9	22.5	40	100
Negative	12	34.2	23	65.8	35	100
Total	43	57.3	32	42.7	75	100

31 (77.5%) of patients with positivity of both markers turned to be positive by histopathology, notably, 12(34.2%) of those considered as no-cancer patients by histopathology have scored high levels of both markers. These findings exposed specificity of 65.7% sensitivity of 77.5%, in addition to positive and negative predictive values of 72% and 71.8% correspondingly.

Discussion

Breast cancer remains the most common cancer in women (excluding skin cancer)[14]. Mortality rates have begun to decrease in the past decade, most likely because of better treatments, earlier detection, and improved screening methods. However, the objective of this study was to evaluate the reliability of circulating tumor markers (CA15-3 & CEA)

in screening for breast cancer, using histopathology as gold standard.

CA15-3

Our findings in this study give a considerable importance for CA15-3 as, a marker that can predict breast cancer (P< 0.001), with high specificity 83.3% and sensitivity of 70%. These findings supported many studies that denoted the reliability of CA15-3 in detection of breast cancer. A significant positive association between CA15-3 concentrations and tumor stage was reported[8,15,16]. Preoperative serum concentrations of CA 15-3 appeared to have a significant relation to outcome in patients with early breast carcinoma.

CA 15.3 has a higher sensitivity in early diagnosis of relapse, as well as being a useful tool for the early diagnosis of metastases, as it is the first indicator of recurrence in 69.5% of patients with relapse (76.3% in patients with metastases)[17]. Furthermore, an initially elevated CA 15-3 level is associated with a very poor prognosis in both early and late stage diseases[7].

As indicated in the results, 12(30%) of the cancer cases showed low levels of CA15-3, also, 5 (14.3%) of those with benign diseases revealed elevated CA15-3 levels as indicated in Table.2. However, these discrepancies were expected as there are several abnormal conditions, other than breast cancer that may lead to high CA15-3 levels[18-20].

CEA

CEA is produced by breast cancer tissue and is then secreted in blood and various body fluids. Breast adenocarcinoma sections stained with monoclonal anti-CEA antibody, exhibited increased CEA production, which is reflected by elevated serum CEA levels[9]. In this study, CEA, scored considerable measures in tern of specificity (80%) and sensitivity (60%), in addition to positive and negative predictive values of 77.4%and 63.6 respectively. These findings exposed a reasonable evidence for the application of CEA as a marker that can predict breast cancer and this was also found to be statistically significant (P< 0.05). However, many studies have reported various specificities, sensitivities and predictive values for CEA. Omar et al.[21]reported 66% sensitivity and 93% specificity with CEA as a diagnostic test for malignancy of breast, whereas, AI Jarallah et al[22]. found a sensitivity of 70% and positive predictive value of 91%. Naghshvar, et al.[23] reported sensitivity and specificity of CEA are 48% and 83%, respectively. These variations may be attributed to the tumor stage and status.

This marker failed to detect 16(40%) of the positive cases, and was elevated in 7(20%) of those with benign diseases and in 2(40%) of the apparently healthy controls. These results were expected, since elevated CEA levels can occur in patients with a number of benign diseases, such as cirrhosis, pulmonary emphysema, rectal polyps, benign breast disease and ulcerative colitis[18].

Regarding, the disagreement between the CEA and histopathology in 16 (40%) cases -negative by CEA and confirmed to be positive by histopathology-, the low sensitivity and specificity of CEA might have contributed to that[19].

The two controls with high CEA levels were smokers, and this can explain the high level of this marker[24].

It is accepted that assays detecting the CA15-3 are more sensitive than those detecting CEA and are more often used in diagnosing this disease, but the combined use of the two types of assays can be more effective[11, 12].

However, specificity of 85.7% and 80% as well as a sensitivity of 70% and 60%, as well as positive predictive values of 85% and 77.4% were credited for CA15-3 and CEA respectively. Likewise, negative predicative value of 71.4% and 63.6% was obtained by CA15-3 CEA. These findings proved that CA15-3 has better specificity and sensitivity than CEA. They also supported a number of studies, revealing that CA15-3 has superior sensitivity compared with CEA, and are more often expressed in breast cancer, but the combined use of the two types of assays can be more effective[17,25,26]. This is strongly supported by our findings.

Moreover, CA 15-3 is one of the first circulating prognostic factors for breast cancer. Preoperative concentrations thus might be combined with other existing prognostic factors for predicting outcome in patients with newly diagnosed breast cancer[10]. A combination of CEA and CA15-3 has been reported to improve lead detection time in metastatic disease, more so than the use of either assay alone[11,27,28].

Conclusion:

In conclusion; the combined use of circulating tumor marker (CA15- &CEA) can emerge as a reliable screening test for breast cancer.

Performing circulating tumor markers (CA15-3 & CEA) is less invasive procedure and is relatively cheap, therefore might be appropriate within a breast-screening program in developing countries.

References:

1. Radiation Isotope Centre Khartoum (RICK), Sudan. Medical records 2002.
2. Gizira Isotope Centre for Molecular biology (GICMB), Sudan. Medical records, 2002.
3. Pons A, Krebs B, Mira R et al. Value of CA15-3 in the follow-up of breast cancer patients. Br J Cancer 1987;55:567-69.
4. Awadelkarim KD, Aceto G, Veschi S et al. BRCA1 and BRCA2 status in a Central Sudanese series of breast cancer patients: interactions with genetic, ethnic and reproductive factors. Breast Cancer Res Treat 2007;102(2):189-99.
5. Colomer R, Ruibal A, Salvador L. Circulating tumor marker levels in advanced breast carcinoma correlate with the extent of metastatic disease. Cancer 1989; 64:1674-1681.
6. Lauro S, Trasatti L, Bordin F et al. Comparison of CEA, MCA, CA 15-3 and CA 27-29 in follow-up and monitoring therapeutic response in breast cancer patients. Anticancer Res 1999;19(4C):3511-5.
7. McLaughlin R, McGrath J, Grimes H **et al**. The prognostic value of the tumor marker CA 15-3 at initial diagnosis of patients with breast cance. Int J Biol Markers 2000;15(4):340-2.
8. Shering S SF, Mcdermott E, O'higgins N **et al**. Preoperative CA 15-3 concentrations predict outcome of patients with breast carcinoma. Cancer 1998; 83(12):2521-7.
9. Murray A WP, Price M, Dixon A **et al**. Evaluation of the IMMULITE BR-MA and CEA assays and comparison with immunoradiometric assays for CA15-3 and CEA in breast cancer. Anticancer Res 1997;17:1945-50.
10. Duffy M. Serum tumor markers in breast cancer: are they of clinical value? Clin Chem 2006;52(3):345-51.
11. Jger W, Kr"Mer S, Palapelas V et al. Breast cancer and clinical utility of CA15-3 and CEA. Scand J Clin Lab Invest 1995;55(221):87-92.
12. American Society Of Clinical Oncology. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. J Clin Oncol 1996;14:2843-77.
13. Bancroft J, Gamble M. Theory and practice of histological techniques. In. London: Churchill Livingstone; 2002;127.
14. Ruibal A, Colomer R, Genolla J. Pronostic value of CA15-3 serum level in patients having breast cancer. Horm Metab 1987;1:11-5.
15. Tampellini M, Berruti A, Gerbino A et al. Relationship between CA15-3 serum levels and disease extent in predicting overall survival of breast cancer patients with newly diagnosed metastatic disease. Br J Cancer 1997;75:698-702.
16. Krebs B, Pons A, Ramaioli A et al. Utilit? du CA15-3 dans le cancer du sein. Cancer commun 1988;2:55-64.
17. 16.Molina R, Jo J, Filella X et al. C-erbB-2, CEA and CA 15.3 serum levels in the early diagnosis of recurrence of breast cancer patients. Anticancer Res 1999;19(4A):2551-5.
18. Jessup J, Thomas P. Carcinoembryonic antigen: function in metastasis by human colorectal carcinoma. Cancer Metastasis Rev 1989;8:263-80.
19. Lucha PA Jr, Rosen L, Olenwine JA et al. Value of carcinoembryonic antigen monitoring in curative surgery for recurrent colorectal carcinoma. Dis Colon Rectum 1997;40:145-9.
20. Symeonidis A, Kouraklis-Symeonidis A, Apostolopoulos D et al. Increased serum CA-15.3 levels in patients with megaloblastic anemia due to vitamin B12 deficiency. Oncology 2004;67(5-6):359-67.
21. Omar Y, Behbehani A, Al Naqueeb N. Carcino-embryonic antigen and breast carcinoma antigen (CA 15-3) in preoperative staging and postoperative monitoring of patients with carcinoma of the breast. Int J Biol Markers 1988;3(3): 165-171.
22. Ai-Jarallah M, Behbehani A, El-Nass S. Serum CA 15-3 and CEA patterns in postsurgical follow-up and in monitoring clinical course of metastasis cancer in patients with, breast carcinoma. Eur J Surg Oncol 1993;19:174-79.
23. Naghshvar F, Torabizadeh Z, Emadian O et al. The diagnostic value of blood level of CEA and CA15-3 tumor markers in breast tumor with axillary lymph node metastases. Journal Of Mazandaran University Of Medical Sciences 2007;16(56):16-20.
24. Cooper E, De Mello Jj, Giles G. Biochemical markers in gastrointestinal malignancies. Arq Gastroenterol 1989;26(4):131-40.
25. Hayes D. Serum (circulating) tumor markers for breast cancer. In. Perry MC (ed) American Society of

Clinical Oncology (ASCO) Educational Book Thirty-third Annual Meeting. 1997:228-233.
26. Canizares F, Sola J, Pérez M et al. Preoperative values of CA 15-3 and CEA as prognostic factors in breast cancer: a multivariate analysis. Tumour Biol 2001;22(5):273-81.
27. Briasoulis E, Andreopoulou E, Tolis C et al. G-CSF induces elevation of circulating CA 15-3 in breast carcinoma patients treated in an adjuvant setting. Cancer 2001;91(5):90.17-9.
28. Kurebayashi J, Nomura T, Hirono M et al. Combined measurement of serum sialyl Lewis X with serum CA15-3 in breast cancer patients. Jpn J Clin Oncol 2006;36(3):150-3.