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Evaluation of CEA and CA15.3 as circulating tumor markers in breast cancer patients in and around coimbatore

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ABSTRACT

Tumor markers are biochemical signs of tumor existence and consist of cell surface antibodies, cytoplasm proteins, enzymes and hormones. The present study was performed to compare the level of CEA and CA15.3 as circulating tumor markers in breast cancer patients. Among 97 breast cancer subjects selected for the study, the levels of CEA and CA15.3 were observed in various stages of breast cancer. Mean level of CEA and CA15.3 were quantitatively determined by EIA. The finding revealed a statistical ($p<0.05$) elevation in the level of CEA in stage IV (100.00%) followed by stage III (53.84%) and stage II (33.33%). On comparison, the CA15.3 is considered to be the best to detect the early stages of breast cancer than CEA. Measuring the levels of CA15.3 and CEA can be helpful for early diagnosis.

KEYWORDS

Breast cancer;
Tumor markers;
CEA;
CA15.3;
EIA.

INTRODUCTION

CA15.3 identified in the sera of breast cancer patients by the use of monoclonal antibody 115D8, is originating from human milk globule membranes. Subsequent studies have used both monoclonal antibodies DF3 and 115D8 in a bideterminant immunoradiometric assay, which has identified a circulating antigen, designated CA15.3[1-3].

Carcinoembryonic antigen (CEA) is a tumor marker produced in the fetus before birth, however its production stops after birth, it does not exist in normal adults. CEA is an example of a molecule expressed at the wrong time because it is normally expressed in significant amounts only during embryonic life. In adults, CEA is only expressed in small amounts in the large intestine[4].

CEA is a normal cell glycoprotein over expressed by several adenocarcinomas, and CA15.3 is a mucin like membrane glycoprotein released by the tumor into the blood stream. CEA and CA15.3 or other MUC-1 products are related to tumor stage, with significant higher values in patients with nodal involvement in patients with larger tumors[5-8].

According to Weinberg[9] two types of markers, (i) oncogene and suppressor gene mutations and (ii) oncogene products may prove to be clinically useful. A number of breast cancer markers have been evaluated. These include CA27.29 (a member of the MUC-1 gene family), carcinoembryonic antigen (CEA), oncoproteins, milk proteins and cytokeratins. The sensitivity and specificity of other members of the MUC-1 gene family such as MCA, CA549, CA15.3 and BRMA are similar to

that of CA27.29[5,9].

In various markers, the best single and established marker for breast cancer is CA13.3, followed by CEA[10]. Nevertheless, the American Society of Clinical Oncology has stated in the Clinical Practice Guidelines for the use of tumor markers that neither CA15.3 nor CEA is recommended for routine use for diagnosing breast cancer.

Breast cancer is a progressive disease; therefore, small tumors are probably diagnosed with tumor markers and treated more successfully[11-12]. The present study is therefore carried out to comparatively state the ability of tumor markers to detect recurrent disease preclinically.

EXPERIMENTAL

Study population

The study subjects were selected from female patients attending the Oncology Departments of Tertiary Hospitals in and around Coimbatore City. 97 subjects who had reported to the clinic during the study period 2004-2008 were screened. Equal numbers of mentally normal, physically healthy females were used as controls. The experimentals were grouped depending upon their stage such as stage I, stage II, stage III and stage IV.

Blood sample collection

5.0 ml of blood samples were collected by venepuncture from subjects and controls aseptically by using heparinised polypropylene tubes. The tubes were immediately placed vertically in a sterile ice packed plastic containers to carry out various hematochemical analyses.

Quantitative determination of CA15.3

CA15.3 was quantitatively determined by Enzyme Immuno Assay[13]. Clotted venous blood sample was centrifuged (REMI centrifuge) and clear serum was collected. The patient and control serum samples were diluted with 1.0 ml of sample diluent. 200µl of CA15.3 standards, diluted specimens and diluted controls were dispensed into appropriate wells and were gently mixed for 10 seconds. After 1-hour incubation at 37^0C the mixture was removed and the micro titer plates (Invitrogen) were rinsed 5 times with deionised water. All the residual water droplets were removed prior to dispensing 200µl of enzyme-conjugate reagent (Invitrogen) into each well. After 10 seconds gentle mixing; it was incubated at 37^0C for 1 hour. The plates were washed after removing the incubation mixture and 100µl of TMB reagent (Invitrogen) was added; mixed for 10 seconds and incubated for 20 minutes in dark, at room temperature. After adding 100µl of stop solution (Invitrogen) to each well, it was mixed for 30 seconds till the blue color changes into yellow and the optical density at 450 nm was read using micro titer plate reader (Merck**)** within 15 minutes.

Quantitative determination of CEA

Enzyme Immuno Assay (EIA) was used to quantitatively determine CEA[14]. Serum was obtained by centrifuging venous blood after clotting. 50µl of standards and test serum were dispensed into assigned wells followed by 100µl of Anti CEA HRP conjugate (Invitrogen). It was thoroughly mixed for 30 seconds and the plates were incubated at room temperature (20-25^0C) for 60 minutes. After incubation, the contents of the well were discarded followed by washing with 300µl of distilled water per well for 5-10 minutes. All residual water droplets were removed by sharply striking the wells onto paper towel. 100µl of substrate solution (TMB) was added to each well, mixed gently for 5 seconds and incubated in the dark for 20 minutes at room temperature (20-25^0C). Later stop solution was added to stop the reaction, mixed for 30 seconds till the blue color changes to yellow and the optical density at 450nm was taken immediately.

RESULTS

According to the stages of breast cancer, 97 study subjects were grouped into 4 based on their stages of breast cancer. Among them 1 subject was found with breast cancer stage I. 12 breast cancer subjects with stage II, 78 and 6 subjects with breast cancer stages III and IV respectively (TABLE 1).

The distribution of CA15.3 levels based on the different stages of breast cancer is represented in TABLE 2. According to the stages of breast cancer, stage IV (40.7 ± 6.34 µg/ml [100.00%]) and stage III ($35.3 \pm$

TABLE 1: Characteristics of patients studied in breast cancer with different stages

Sl. no	Breast cancer stages	Number of patients	Percentage of patients
01	Stage I	1	0.97%
02	Stage II	12	11.64%
03	Stage III	78	75.66%
04	Stage IV	6	5.82%

Stage I: Tumor is less than 2 cm across and hasn't spread beyond the breast, Stage II: Tumor is less than 2 cm across and has spread to the lymph nodes, Stage III: Tumor is greater than 5 cm across and has spread to lymph node, Stage IV: Metastatic breast cancer

TABLE 2: Distribution of CA15.3 levels according to breast cancer stages

Breast cancer stages	Total number of cases (n=97)	Cases with CA15.3 levels (>30μg/ml)	Mean±SEM
Stage I	1	-	-
Stage II	12	5 (41.66)	19.7 ± 1.63
Stage III	78	56 (71.79)	35.3 ± 4.08*
Stage IV	6	6 (100.00)	40.7 ± 6.34*

*** p<0.05**

TABLE 3: Distribution of CEA levels according to breast cancer stages

Breast cancer stages	Total number of cases (n=97)	Cases with CEA levels (>5.0ng/ml)	Mean ± SEM
Stage I	1	-	-
Stage II	12	4 (33.33)	8.62±1.43
Stage III	78	42 (53.84)	10.89±1.04*
Stage IV	6	6 (100.00)	14.80±2.29*

*** p<0.05**

4.08 μg/ml [71.79%]) shows a statistically significant ($p<0.05$) increase than stage II (19.7 ± 1.63 μg/ml [41.66%]) respectively. The values show a sequential significance based on the breast cancer stages.

TABLE 3 represents the distribution of CEA levels according to the breast cancer stages. The mean levels of CEA shows a statistically significant increase in stage III 10.89 ±1.04 ng/ml (53.84%), followed by stage IV 14.80 ± 2.29 ng/ml (100.00%) when compared to stage II 8.62 ± 1.43 ng/ml (33.33%).

DISCUSSION

Tumor markers are expected to play a role in the differential diagnoses, early detection of cancer, prognostic predictions, monitoring of treatment efficacy and surveillance of disease relapse[10].

In breast cancer, however, the roles of serum markers are less well established. The most widely used serum markers in breast cancer are CA15.3 and carcinoembryonic antigen (CEA)[15].

In healthy subjects, the upper limit of the normal CA15.3 concentration is 30U/mL. In the present study, the distribution of CA15.3 levels of stage IV (100.00%) and stage III (71.79 %) showed a statistically significant increase than stage II (41.66%). There was no detectable amount of CA15.3 in stage I. The mean level of CA15.3 in breast cancer cases revealed a sequential significant elevation based on the stages. CA15.3 has been shown to be elevated in 95% of cases where metastasis existed[16]. CA15.3 concentrations are increased in 10% of patients with stage I disease, 20% with stage II disease, 40% with stage III disease and 75% with stage IV disease. In contrast, more than 70% of patients with distant metastasis have elevated marker concentrations. Concentration can be particularly high when either bone or liver metastasis is present[17,18].

Some studies show that CA15.3 increases rarely in the early stages of breast cancer[19], while others indicated that it often increases[20]. It may increase in pancreas cancer as well as in spleen cancers. It may increase in non-malignant cases in hepatitis and cirrhosis as well[21].

The use of CA15.3 for early detection of metastasis seems to be promising[22]. They are widely used in measuring therapeutic response in metastatic diseases[23]. Breast cancer patients who received G-CSF (Granulocyte Colony Stimulating Factor) primed chemotherapy showed serum CA15.3 elevation due to an increase in peripheral blood neutrophil number and induced neutrophil cytoplasmic MUC1 expression which was caused by G-CSF[24] .

According to an American Society of Clinical Oncology (ASCO) expert panel, CA15.3 concentration at 5 to 10 fold above the upper limit of the reference interval could alert the presence of metastatic disease. However, a low concentration does not exclude metastasis. As CA15.3 concentrations are elevated in majority of the breast cancer patients with distant metastasis, it might appear reasonable to use this marker to monitor response to treatment therapies and recommended the use of CA15.3 for monitoring therapy in patients with advanced breast cancer[25].

In the present study, the distribution of mean CEA

levels in the breast cancer patients has statistically significant increase in stage IV (100.00%) followed by stage III (53.84%), when compared to stage II (33.33%). CEA values are elevated in approximately 50% of patients with tumor extension to lymph nodes and 75% of patients with distant metastasis. The highest values (above 100ng/ml) occur with metastasis[26], although poorly differentiated tumors are less likely to produce CEA[27].

Non-neoplastic conditions associated with elevated CEA levels include cigarette smoking, peptic ulcer disease, inflammatory bowel disease, pancreatitis, hypothyroidism, biliary obstruction and cirrhosis. Levels exceeding 10ng/ml are rarely due to benign disease[28].

CEA and CA15.3 are used to follow up the breast cancer[29-34]. These tests are not usually used to monitor a primary cancer diagnosis[33]; or to get to an early diagnosis of recurrence and metastasis[35].

Between the two markers, CA15.3 is considered to be the best to detect the stages of breast cancer than CEA in 97 breast cancer patients studied. Similar studies were performed in America and Taiwan where CA15.3 has been introduced as a marker better than CEA to assess and prognoses the treatment results in women affected with breast cancer[36]. A sensitivity of 39% for CEA and 65% for CA15.3 was reported in predicting metastasis[39]. Combination of two markers showed a sensitivity of 69%. CA15.3, which in many instances is a better tumor marker than CEA in breast cancer[37]. The incidence of plasma marker elevation in advanced breast cancer is 69% for CEA and 89% for CA15.3[37].

When compared the clinical stages of serum levels of CEA and CA15.3, the CA15.3 was more sensitive and specific in metastatic breast cancer than CEA[33,35]. These findings support our results.

Tumor marker sensitivity in patients with advanced breast cancer is significantly higher than in those with localized or regional disease[6,38]. In recent decades, tumor markers such as CEA and CA15.3 have been used as a warning sign of distant metastasis of breast cancer[39-43].

Indeed the use of markers to monitor therapy has several advantages over conventional criteria, including increased sensitivity, more objective measurement and more convenience for patients[44,45].

Although available data show relatively good correlations between alterations in serial tumor marker concentrations and response to therapy in advanced breast cancer, neither CA15.3 nor CEA should be routinely used for this purpose[27].

The availability of a reliable blood test could enable implementation of circulating tumor cells as a surrogate marker for clinical development of new anticancer agents and optimization of existing treatment protocols[46]. One of the main purposes of measuring tumor markers was to monitor the outcome of metastases in breast cancer patients[47].

From this study, it is observed that the level of CA15.3 and CEA is significantly higher in breast cancer patients. CA15.3 was found to be more sensitive in metastatic stages of cancer. This study, though of small size suggests that CA15.3 can be used as a tumor marker for detecting early stages of breast cancer. However, the exact reasons for the elevated level of CA15.3 in breast cancer metastasis need to be investigated in detail.

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