CORRELATION STUDY OF CARCINO EMBRYONIC ANTIGEN & CANCER ANTIGEN 15.3 IN PRETREATED FEMALE BREAST CANCER PATIENTS

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ABSTRACT

Carcino Embryonic Antigen (CEA) and Cancer Antigen 15.3 (CA15.3) are the most common tumor markers in breast cancer patients. Measurement of circulating tumor markers is a non-invasive quantitative method. Serum levels of CEA and CA15.3 were studied in female breast cancer patients prior to treatment. To evaluate the utility of these markers, 207 Breast carcinoma patients belonging to all the stages were considered. Healthy age matched 75 female individuals formed the control group. The serum levels of CEA and CA 15.3 were analyzed by Enzyme Linked Immunosorbent Assay (ELISA). Results were taken and compared with stages, tumor size, node and grade. The serum CA15.3 levels were significant in all the study parameters whereas serum CEA levels showed no significant changes with any of the parameters. Measurement of serum CA15.3 levels showed significant correlation (24.8%) with advanced stages and larger tumor sizes, whereas serum CEA levels did not show any significant correlation in breast cancer patients prior to treatment.

KEY WORDS

Breast cancer, Tumor markers, stage, Tumor size, Node, Grade

INTRODUCTION

Adenocarcinoma of the breast is the most common cancer. Carcinoembryonic Antigen (CEA) and cancer Antigen 15-3 (CA15.3) are the most thoroughly investigated tumor markers in breast cancer. Circulating levels of CEA and CA15.3 have become established diagnostic tools as fast, non-invasive, reproducible, quantitative parameters in follow up care and monitoring therapy of breast cancer patients.

Carcinoembryonic antigen (CEA) was first described in 1965 by Gold and Freedman (1), because the protein was detected in only cancer and embryonic tissue, it was given the name carcinoembryonic antigen, or CEA. CEA is a member of the immunoglobulin superfamily. The human CEA gene family is clustered on chromosome 19q and comprises 29 genes. Of these, 18 are expressed, with 7 belonging to the CEA subgroup and 11 to the pregnancy-specific glycoprotein subgroup (2).

When isolated from liver metastasis, CEA is a glycoprotein consisting of ~ 60% carbohydrate and a molecular mass of ~ 180–200 kDa. Most of the carbohydrate is composed of mannose, galactose, *N*-acetylglucosamine, fucose, and sialic acid (3).

CA 15.3 assay measures the protein product of the MUC-1 gene located on chromosome 1q. MUC1 protein is a large transmembrane glycosylated molecule containing three main domains, a large extracellular region, a membrane spanning sequence and a cytoplasmic domain.(4,5) The CA15.3 is a glycoprotein, helps in cell adhesion, immunity and metastasis(2). The antigen is defined by reacting with two monoclonal antibodies: DF3 and 115D8. The DF3 antibody was produced against a membrane preparation from a breast carcinoma metastasis. The 115D8 antibody was prepared against milk fat globulin membrane and may be directed to a carbohydrate epitope (6).

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Serum CEA and CA15.3 are established prognostic markers in breast cancer patients. This study is aimed to correlate the serum marker levels with the clinical stage of the disease, size of the tumor, nodal status and grade in pretreated female breast cancer patients. If a correlation is found, it could provide clinicians an indication of prognosis preoperatively, thus

enabling appropriate neoadjuvant therapy.

MATERIALS & METHODS

This study was carried out in the Department of Biochemistry at Kidwai Memorial Institute of Oncology. Patient's data were collected from Breast Service Unit of Surgical Oncology. The study population included 252 newly registered consecutive series of patients in the age group of 26-76 years. Recruitment of patients for this study were registered cases of Nov 2004-October 2005 from Breast Services Unit. Among them, 18 patients had benign breast diseases, 5 were male patients and 22 treated cases. Only 207 pretreated histologically confirmed female breast cancer patients belonging to different stage of the disease and 75 age- matched healthy female controls were selected among volunteers for our study. Since our focus was to study the correlation between serum CEA and CA15.3 in pretreated female breast cancer patients, we excluded 18 benign breast diseases, 5 male patients and 22 treated cases from the study. Patient's disease stage, tumor size, node and grade were noted from the case files. Blood samples were collected in plain tubes, centrifuged to separate the serum. The serum samples were stored at -20°C until analysis (a month). The study was approved by the Institutional Ethics committee. Informed consent was obtained from each patient prior to sample collection.

The markers (CEA, and CA15.3) were analysed by sandwich ELISA method using Calbiotech kits.

CEA : 50 microlitre of the patient's samples and 100 microlitre anti-HRP (horse radish peroxidase) conjugate were added to the microwells coated with monoclonal antibody (MAb). The antibody -enzyme conjugate solution contained goat anti CEA antibody conjugated to HRP. CEA in the patient's serum bound to anti CEA MAb on the well and the anti-CEA-HRP second antibody then bound to CEA. Unbound protein and HRP conjugate was washed off by wash buffer (phosphate buffer saline). Upon the addition of the TMB (3 3' 5 5' tetra methyl benzidine) substrate, the intensity of color is proportional to the concentration of CEA in the samples. The color development was stopped with the addition of stop solution (1N HCl).

CA15.3: CA15.3 ELISA test is based on the principle of a solid phase enzyme linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a antigenic determinant on the intact CA15.3 molecule was used for solid phase immobilization (on the micro wells). The antibody - enzyme conjugate solution contained rabbit anti CA15.3 antibody conjugated to HRP. 200 microlitre diluted

patient's sample (21times) was allowed to react sequentially with the two antibodies, resulting in the CA15.3 molecules being sandwiched between the solid phase and enzyme linked antibodies. After two separate one hour incubation steps at 37°C, the wells were washed with water to remove unbound labeled antibodies. A solution of TMB reagent was added and incubated for 20 minutes, resulting in the development of a blue color. The color development was stopped with the addition of stop solution (1N HCl).

A standard curve was prepared relating color intensity to the concentration of the markers. Statistical significance was tested by using unpaired student's t-test. $P < 0.05$ was considered significant. Regression analysis was carried out for predicting serum levels of CEA and CA15.3 in relation to study parameters. Early stage(I and II), smaller tumor (size<5cm), node negative and lower grade(1 and 2) were kept as reference category for carrying out the regression analysis.

RESULTS & DISCUSSION

Table I shows mean pattern of serum levels of CEA and its relationship with different stages, tumor size, node and tumor grade in breast cancer patients. The serum CEA levels of patients do not show significant change in any of the parameters. Table II shows mean pattern of serum CA15.3 levels with study parameters. Mean and standard error of mean showed significant changes in advanced stages ($P=0.0014$), larger tumor size ($P<0.0001$), positive nodes($P=0.013$) and higher grade($P=0.023$).

Table III shows regression analysis for predicting serum levels of CEA and CA15.3 in relation to study parameters. Serum CEA levels were not associated with any of the parameters

Table I : Mean Pattern of serum CEA and its relationship with study parameters.

Study parameters	Subjects (n)	CEA ng/ml Mean $\pm$ SE	Significance (P)
Stage	Control (75)	3.04 $\pm$ 0.21	0.122
	Stage I&II (81)	4.97 $\pm$ 0.79	
	Stage III&IV(126)	6.86 $\pm$ 0.833	
Tumor size	<5cm(123)	4.106 $\pm$ 0.48	0.263
	$\geq$ 5cm (84)	6.078 $\pm$ 1.02	
Node	Negative (100)	5.58 $\pm$ 0.72	0.09
	Positive (107)	8.64 $\pm$ 1.66	
Grade	1+2(72)	5.16 $\pm$ 0.84	0.517
	3(135)	7.55 $\pm$ 1.09	

Table II : Mean Pattern of serum CA15.3 and its relationship with study parameters

Study parameters	Subjects (n)	CA15.3 IU/L Mean $\pm$ SE	Significance (P)
Stage	Control (75)	13.9 $\pm$ 1.78	0.0014
	Stage I&II (81)	42.35 $\pm$ 3.72	
	Stage III&IV(126)	75.46 $\pm$ 7.86	
Tumor size	<5cm(123)	40.57 $\pm$ 3.05	<0.0001
	$\geq$ 5cm (84)	79.24 $\pm$ 7.84	
Node	Negative (100)	36.22 $\pm$ 4.15	0.013
	Positive (107)	62.65 $\pm$ 8.69	
Grade	1+2(72)	43.77 $\pm$ 4.75	0.023
	3(135)	66.43 $\pm$ 6.68	

and regression value ($R^2=11.4\%$) was low. Serum CA15.3 levels were associated with advanced stages and larger tumor sizes ($\geq 5\text{cm}$). The regression value ($R^2=24.8\%$) for serum CA15.3 was significantly ($P<0.001$) higher when compared to serum CEA levels.

CA 15.3 is one of the first circulating markers shown to provide independent prognostic information in patients with breast

Table III : Regression analysis for predicting serum levels of CEA and CA15.3 in relation to study parameters

Predictors	Serum CEA		Serum CA15.3	
	Regression co-efficient	P value	Regression co-efficient	P value
Stage III & IV	3.198	0.11	82.22	0.001
Tumor size	0.486	0.256	19.218	0.038
Node +	1.575	0.386	3.313	0.129
Grade 3	-2.626	0.405	0.642	0.69
R^2 (P value)	11.4%(0.18)		24.8% (<0.001)	

carcinoma. CA 15.3 should be a relatively noninvasive and inexpensive approach to obtain prognostic information in newly diagnosed breast cancer patients (7). Elevated serum levels of CA 15.3 are found more in breast carcinoma patients with distant metastases, although the marker is rarely elevated in patients with local or primary breast carcinoma(8). Currently, the main clinical application of CA 15.3 is for monitoring patients with diagnosed breast carcinoma and preclinically detecting recurrences (9). Giai et al (10) demonstrated the usefulness of this marker in facilitating the selection of patients who may require additional therapy preoperatively. CA15.3 can be utilized as a useful prognostic marker, particularly in cases where histopathological variables such as tumor size

and grade have been altered by neoadjuvant chemotherapy (11). The use of CA15.3 for early detection of metastases seems to be promising (12) and their use for measuring therapeutic response in metastatic disease is widely accepted (13). Women with resected breast carcinoma who received G-CSF (Granulocyte Colony Stimulating Factor) primed chemotherapy showed serum CA 15-3 elevation due to an increase in peripheral blood neutrophil number and induced neutrophil cytoplasmic MUC1 expression which was caused by G-CSF (14). The study by Safi et al (15) showed that CA15.3 was significantly better than CEA in the detection of breast cancer metastases. It is generally agreed that tumor markers in breast cancer patients are not a tool for primary diagnosis because of their low sensitivity and specificity (16). The increased CA15.3 values when combined with increased serum HER-2/Neu (human epidermal receptor -2/neu) predict a worse prognosis than only increased CA15.3 value (17).

A College of American Pathologists Expert Groups ranked preoperative serum CEA concentration as a category I prognostic marker for colorectal cancer (18). CEA exhibits relatively poor sensitivity for the detection of locoregional recurrences, i.e., ~60% (19). Despite this limited sensitivity for locoregional disease, Pietra et al. (20) showed in a prospective randomized trial that CEA was superior to endoscopy, computerized tomography, and ultrasound in diagnosing local recurrences. Serial CEA determinations are most useful in detecting liver metastasis. Using 196 patients, CEA was reported to have a sensitivity of 100% in detecting metastatic liver disease (20).

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