RESEARCH ARTICLE

Relationship between Preoperative Serum CA15-3 and CEA Levels and Clinicopathological Parameters in Breast Cancer

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Abstract

Background: CEA and CA 15.3 serum tumor markers are currently used in clinical practice for monitoring therapy. The aim of this study was to evaluate serum level of these markers among healthy females and invasive breast carcinoma (IBC) patients and to determine any relationships with clinicopathological factors. Materials and Methods: 60 Iranian females were enrolled in this study, 30 healthy and 30 diagnosed with breast cancer who had not received any preoperative chemotherapy or hormone therapy. Enzyme linked immunosorbent assays were used for the quantitative determination of the cancer associated antigens, CEA and MUC1 (CA15-3). Results: The serological levels of CEA and CA15-3 (5.0033±0.49 μg/L and 178.1667±15.11 U/ml) in the breast cancer patients were significantly higher (p=0.00) than the serum levels of normal controls (1.1237±0.11 μg/L and 21.13±3.058 U/ml). Regarding the CEA marker, a significant correlation with grade of tumor was shown. Furthermore, there was a low correlation between CA15-3 and CEA marker with correlation coefficient r=0.08. Conclusions: Collectively, markedly high levels of CEA and CA15-3 were found in our patients, pointing to their use as additional tools after clinical diagnosis.

Keywords: Serum tumor marker - invasive breast carcinoma - clinicopathological factors

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Introduction

In Iran, 10,000 women are diagnosed each year with breast cancer (Mousavi et al., 2009; Zare et al., 2012). Research on diagnostic or prognostic markers in breast cancer is important and essential, considering the fact that trend of breast cancer mortality is increasing.

Detection of serum tumor marker as easily accessible and soluble circulating markers in females with breast cancer is a useful strategy for evaluation of prognosis and selection the type of treatment.

The MUC1 gene encodes high molecular weight, mucin glycoproteins that are normally expressed on the apical surface of mammary epithelial cells including breast cells (Duffy, 1999). This marker plays the biological roles such as cell adhesion, immunity and it is responsible for metastasis (Thriveni et al., 2007). However, MUC1 is overexpressed and improperly glycosylated in malignant epithelial cells especially tumor cells (Barros et al., 1994). The protein product of the MUC1 gene was determined by serum tumor marker CA15-3 assay via monoclonal antibodies that stick to epitopes on the MUC1 molecule.

CEA stands for carcinoembryonic antigen and it is the member of the immunoglobulin superfamily (Thomas et al., 1990). This protein molecule can also be detected in various cells of the body, but it is frequently associated with distinctive tumors, so it has a significant role as a tumor marker (Duffy, 1989).

In this project, levels of CEA and CA15-3 markers in serum of breast cancer patients was compared with level of these markers in serum of healthy controls. Relation of the level of CEA and CA15-3 markers and clinicopathological factors such as lymph node position, tumor grade, stage and size of tumor, age of patients and protein receptors status were investigated.

Materials and Methods

Clinical assessment was carried out in 30 female breast cancer patients based on histological reports at different stages in the age group of 23-87 years who had not received any preoperative chemotherapy or hormone therapy from May 2012 to March 2013 at the Milad hospital, Tehran. Breast cancer staging (I–IV) classified according to standard criteria based on data of TNM (Tumor, Nodes and Metastases) and American Joint Committee on cancer staging system (AJCC). 30 age-matched healthy female subjects with no history of breast cancer were taken as control.

Sample collection

Sample collection and subsequent use were accomplished according to the permission from National Ethical Committee from Pasteur Institute of Iran. Before blood collection a written consent form was signed by each subject. Peripheral blood samples were collected

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from patients and healthy controls in 10 ml glass tubes without additive; serum was separated by centrifugation (2500 rpm, 10 min) and stored at -20°C for later analysis.

Detection of CEA and CA15-3

The commercially available CanAg CA15-3 and CanAg CEA EIA kits (FUJIREBIO Diagnostics, Inc.) are used for the quantitative determination of the cancer associated antigens in serum. The markers (CEA and CA15-3) were analyzed by direct sandwich technique by two monoclonal antibodies. When the reaction was terminated by a stop solution (0.12 M hydrochloric acid), the absorbance (optical density at 405-630nm) was measured by ELISA reader. The standard curve was prepared based on absorbance.

Statistical analysis

Statistical significance was tested by using unpaired student's t-test. The p value of <0.05 was considered as significant. Serum levels of CEA and CA15.3 in relation to clinical pathology was also considered. Regression analysis was analyzed and as a reference of regression analysis the node negative, smaller tumor size) ≤2cm), lower grade (I and II), early stage (I and IIA) and age <50 was considered.

Results

Clinicopathological characteristic of 30 breast cancer patients was extracted from questionnaire (Table 1). Mean age was 38.92±1.82 and 55.35±2.54 years in pre and post-menopause respectively. Serum CA15-3 levels was 177.92±21.40 and 167.05±17.05 U/ml and CEA levels was 4.36±0.67 and 5.49±0.7 μg/L in pre and post-menopause respectively. 28 patients (93.3%) were diagnosed histologically as ductal infiltrating carcinoma while 2 patients (6.6%) were diagnosed as invasive lobular carcinoma. Tumor size was classified as T1 (Tumor size less than or equal to 2 centimeters) in 5(16.6%), T2-T3 (Tumor size between 2 and 5 centimeters) in 24(80%) and T4 (Tumor extends to chest wall) in 1(3.3%) of cases according to the TNM classification was observed. Molecular classification based on the pathological criteria of breast cancer was done. Out of 30 cases, 2 patients (6.6%) were classified as Basal like, 12 patients (40%) as Luminal category, 5 patients (16.6%) as Her2-enriched and 7 patients (23.3%) were considered as Triple-positive.

Abnormal CEA (>2μg/L) or CA15.3 (>30U/mL) serum levels were detected in 90% and 96.6% of the patients studied, respectively. One or both of the markers were abnormal in 100%.

The mean levels of Serum CEA and CA15-3 among healthy and breast cancer groups were displayed in Table 2. The healthy groups have value of tumor markers CEA 1.1237±0.11 μg/L and CA15-3 21.13±3.058 U/mL. Elevated serum level of CEA (5.0033±0.49 μg/L) and CA15-3 (178.1667±15.11 U/ml) markers was seen among breast cancer patients and it was statistically significant (p=0.00).

Table 3 demonstrates mean pattern of serum levels of CA15-3 and its relationship with study parameters. Based on tumor size, patients with malignant breast lesions were categorized into two groups, ≤2 cm and >2 cm. Serum CA15-3 was statistically significant higher among breast

Table 1. Clinical-Pathological Characteristics

Characteristic			N	%
Patients			30	100
Mean age=48 (range 23-87 years)				
Premenopausal			38.92±1.82	
Postmenopausal			55.35±2.54	
Menopausal status				
Pre			13	43.3
Serum CA15-3(U/ml)			177.92±21.40	
Serum CEA(μg/L)			4.36±0.67	
Post			17	56.6
Serum CA15-3(U/ml)			167.05±17.05	
Serum CEA(μg/L)			5.49±0.7	
Surgery	Modified Radical mastectomy		7	23.3
	Mastectomy		7	23.3
	Partial mastectomy		11	36.6
	Unknown		5	16.6
Site of cancer	Right		12	40
	Left		18	60
Histological diagnosis ductal				
	IDC		21	70
	NOS Type		7	23.3
	ILC		2	6.6
Tumor size (T)		T1	5	16.6
		T2	22	73.3
		T3	2	6.6
		T4	1	3.3
Immunohistochemical profile		Basal like	2	6.6
		Luminal category	12	40
		Her2-enriched	5	16.6
		Triple-positive	7	23.3
		Unknown	4	13.3

*CA15-3: Cancer antigen 15-3 (U/ml), CEA: Carcino Embryonic antigen (μg/L), N: number of subjects; Basal like: triple negative (ER/PR/Her2-negative); Luminal category: ER/PR-positive and Her2 negative; Her2-enriched: ER/PR-negative and Her2 positive; Triple-positive: ER/PR/Her2-positve; **Invasive Ductal Carcinoma: IDC; Invasive Ductal Carcinoma: NOS Type; Invasive Lobular Carcinoma: ILC

Table 2. Mean Pattern of Serum CEA and CA15-3 Levels in Study Groups

	N	%	Mean±SEM Ag CEA	p value	Mean±SEM Ag CA15-3	p value
Healthy control females						
	30	50	1.1237±0.11		21.13±3.058	
Breast cancer patients						
	30	50	5.0033±0.49	0.00*	178.1667±15.11	0.00*
All groups	60	100				

*CA15-3: Cancer antigen 15-3 (U/ml); CEA: Carcino Embryonic antigen (μg/L); N number of subjects; SEM: standard error of mean; p<0.05 between healthy control and breast cancer patients

Table 3. Relationship between Study Parameters and CA15-3 Molecular Marker

Study parameters		N (%)	Mean±SEM U/mL	p value
Node position	ve +	14(46)	165.7857±14.72800	0.679
	ve -	16(54)	177.0000±21.51240	
Tumor size	≤2cm	7(23)	229.8571±11.53964	0.012*
	>2cm	23(76)	154.087±15.08093	
Grade	I&II (low)	19(63)	174.1053±18.30475	0.82
	III (high)	11(36)	167.7273±18.24335	
Stage	I&IIA	18(60)	178.3889±19.32169	0.548
	IIB, III& IV	12(40)	161.8333±16.34701	
Age	<50	17(56)	173.8235±19.99122	0.862
	≥50	13(44)	169.0769±16.52793	

Table 4. Relationship between Study Parameters and CEA Molecular Marker

Study parameters		N (%)	Mean±SEM	p value
Node position	ve+	14(46)	5.4857±0.88832	
	ve-	16(54)	4.5812±0.52299	0.374
Tumor size	≤2cm	7(23)	6.4143±1.13650	
	>2cm	23(76)	4.5739±0.53151	0.119
Grade	I&II(low)	19(63)	4.2263±0.50702	
	III (high)	11(36)	6.3455±0.93501	0.038*
Stage	I&IIA	18(60)	5.3500±0.68938	
	IIB, III& IV	12(40)	4.4833±0.69922	0.403
Age	<50	17(56)	4.7824±0.70779	
	≥50	13(44)	5.2923±0.70342	0.62

Table 5. Regression Analysis of CEA and CA15.3 Serum Levels in Relation to Study Parameters

Predictors	CEA		CA15.3	
Independent Variables	Regression Co-efficient	p value	Regression Co-efficient	p value
Stage IIB, III& IV	-0.089	0.842	0.103	0.838
Tumor size>2 cm	-0.058	0.886	0.072	0.764
Node positive (+)	-0.283	0.105	0.115	0.519
Grade III	-0.403	0.093	0.046	0.93
Age>50	-0.402	0.278	0.058	0.903

Table 6. Tissue Marker Status and Serum CA15-3 and CEA Levels in Breast Cancer Patients

STM		N (%)	Mean±SEM CEA	p value	Mean±SEM CA15.3	p value
Her2+	3	12(40)	5.47±0.79		176.5±20.60	
(score)	0 or 1	14(46)	4.62±0.75		170.85±19.81	
Unknown		4(13)		0.45		0.84
P53	+	8(26)	5.33±1.17		170.37±28.7	
	-	17(56)	4.82±0.64		170.41±16.65	
Unknown		5(16)		0.68		0.99
PR	+	17(56)	4.4±0.46		169.29±18.49	
	-	9(30)	6.18±1.26		181.33±21.65	
Unknown		4(13)		0.12		0.69
ER	+	19(63)	4.85±0.56		165.57±17.05	
	-	7(23)	5.47±1.39		194.85±23.79	
Unknown		4(13)		0.62		0.36
Ki67	+	23(76)	4.86±0.60		169.26±14.9	
	-	2 (6)	6.35±0.15		183.5±66.5	
Unknown		5(16)		0.12		0.79
triple-positive (ER/PR/HER2 positive)						
		7(23.3)	4.98±0.72	0.4*	163.14±29.09	0.4*
HER2-enriched (ER/PR negative & HER2 positive)						
		5(16.6)	6.16±1.7	0.48**	195.2±29.58	0.9**
Basal like triple-negative (TN) (ER/PR/HER2 negative)						
		2 (6.6)	3.75±2.75	0.5***	194.0±56.00	0.63***

*PR: Progesterone receptor; ER: Estrogen receptor; HER2: human epidermal growth factor receptor 2; p value<0.05 between hormone receptor (+) compared to hormone receptor (-); *p value between triple-positive (ER/PR/HER2 positive)compared to HER2- enriched (ER/PR negative & HER2positive); **p value between HER2- enriched (ER/PR negative&HER2positive) compared triple-negative (ER/PR/HER2 negative) (TN); ***p value between triple-positive (ER/PR/HER2 positive) compared to Basal like or triple-negative (ER/PR/HER2 negative) (TN)

cancer patients that have tumor size with ≤2 cm (p=0.012).

The serum CA15-3 levels of patients do not represent significant change in other study parameters (node position, grade, stage and age).

In Table 4 mean pattern of serum levels of CEA and its relationship with study parameters was shown. The serological values CEA in the cancer patients with grade III were significantly higher (p=0.038) than the serum levels of cancer patients with grade I&II. There was no significant difference in CEA level among other parameters (node position, tumor size, stage and age).

On the other hand, linear regression analysis of the values for both serum tumor markers was assessed. In this study, correlation of study parameters with CA15-3 and CEA markers was not observed (Table 5). Based on linear regression analysis of the correlation between CEA and CA15.3 serum levels and clinicopathological features, we found that Stage, tumor size, node position, grade and age were not predictive factors for CEA and CA15.3 serum levels (Table 5).

Table 6 summarizes the frequency and percentage of breast cancer patients with positive and negative human epidermal growth factor receptor 2(Her2), p53, progesterone receptor (PR), estrogen receptor (ER) and Ki67.

Based on immunohistochemistry analyses, among 30 valid cases, total number of Her2, p53, PR, ER and Ki67 positive cases was 12 (40%) , 8(26%), 17(56%), 19(63%) and 23(76%) respectively and negative cases was 14(46%), 17(56%), 9(30%), 7(23%) and 2(6%) respectively.

The concentration of serum CA15-3 and CEA was also determined in each group.

The serum CEA and CA15-3 levels of patients were not shown any significant differences between positive and negative immunohistological (Her2, hormone receptors, p53 and ki67) groups. We also not found any significant differences between serum levels of molecular surrogate types, triple-positive (ER/PR/HER2 positive), Her2-enriched (ER/PR negative & Her2 positive) and triple-negative (ER/PR/HER2 negative).

Discussion

In present study, the age distribution of Iranian women suffering from breast carcinoma ranged from 23-87 years and the average age of them was 48 years. The most frequent age was 51 year, 4(12.5%) and 6(20%) patients were under 40 years. Mousavi et al. 2006 showed that more than 36% of the tumors occur in women under the age of 40 years and breast cancer is a high burden in Iran. The mean age of premenopausal and postmenopausal women was 39 and 55 years respectively. We observed a significant increased value of CA15-3 and CEA in both group of pre and postmenopausal patients as compared to healthy control females. However there isn't any significant difference between the levels of serum antigens in premenopausal compared to postmenopausal patients groups. This result is different from other study that it has been carried out on Pakistani females (Begum et al., 2012).

Most of breast cancer patients are diagnosed with invasive ductal carcinoma (Begum et al., 2012) and we also found in our patients, out of 30 patients, 28 patients (93.3%) were invasive ductal carcinoma and only 2 patients (6.6%) were invasive lobular carcinoma.

It has been shown that different hormone receptor status could have an effect on the level of breast cancer patient's mortality (Dunnwald et al., 2007). We found majority of patients, 63% were hormone receptor positive (ER/PR+) and only 6.6% were identified as triple negative. The mean serum CA15-3 and CEA between hormone receptor positive and triple negative groups was not shown any significant difference. However, Bensouda et al., 2009

and Atoum et al., 2012 reported that estrogen receptor status is strongly correlated with elevated CA15-3 level.

Generally, colon cancer procreates a protein identified as Carcinoembryonic Antigen or CEA (Compton et al., 2000). The increase in value of preoperative CEA may be beneficial in predicting the prognosis of patients with colorectal cancer especially combined with CA19-9 (Vukobrat-Bijedic et al., 2012). On the other hand, the CEA is also raised in other forms of cancer (lung, pancreas, stomach, and breast).

In our study, serum CEA was significantly elevated in breast cancer patients than healthy females (p=0.00). Martoni et al., 1995 have reported that serum measurements of CEA were high at patients with adenocarcinoma.

The routine use of serum CA 15-3 as mucinous marker is in monitoring therapy in patients with advanced breast cancer. Its preoperative levels were assessed in judging the diagnosis in Iranian women with breast cancer. In present study, the average concentration of CA15-3 was significantly higher in breast cancer patients than normal's (p=0.00). This finding is consistent with other reports (Keyhani et al., 2005; Agha-Hosseini et al., 2009).

Hence, these tumor markers (CEA and CA15-3) could be used as diagnostic factors in association with clinical diagnosis factors for cancer detection.

linear regression analysis of the values for the tumor markers illustrate that serum CA15-3 values were not correlated with serum CEA values (r=0.08). However, in other investigation groups it was shown good correlation between these tumor markers (Serdarević et al., 2012).

No correlation was found between the age, stage, tumor size and node position of the patients and CEA values. Our result indicated that significant correlation was observed between serum CEA and tumor grading (p=0.038). However, regression analysis showed low prediction. Our findings is the same as the study by Thriveni et al., 2007 that displayed regression analysis for predicting serum levels of CEA and it was also low in relation to stage, tumor size, node and tumor grade. They also displayed that serum CA15-3 levels were associated with advanced stages and larger tumor sizes (>5cm). Therefore, they introduced CA15-3 marker as a good marker for evaluating the progression of breast cancer. However, our study shows no significant association between breast cancer age, stage, node position and tumor grading. However, higher serum level of CA15-3 among breast cancer patients was statistically significant in patients that have tumor size with ≤ 2 cm, while regression analysis showed low prediction for serum levels of CA15-3 in relation to tumor size.

Due to low sensitivity and specificity of tumor markers, they cannot be useful tools for primary diagnosis in breast cancer patients (Tondini et al., 1989) but it could be used as an initial tumor marker in the management of breast cancer patients. On the other hand, association of tumor markers may be raising the sensitivity especially for detection of metastatic breast cancer.

In conclusion, CA15-3 and CEA serum level was shown independent on age, grade, node position, staging, and tumor size or hormone receptor status among breast cancer groups but elevated serum CA15-3 and CEA was found in all breast cancer patients. There is no direct relation between serum level of CA15-3 and CEA and clinicopathology of patients. However, serum levels of these two markers are significantly higher than serum levels of normals and assessment of more population should be considered to find exact relation of these markers to breast cancer.

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