

Correlation of Ca15-3 and CEA in Different Molecular Subtypes of Metastatic Breast Cancer

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ABSTRACT

Introduction: Breast cancer is the most common cancer occurring in woman worldwide. Nearly 25% of all cancers is breast cancer with 1.67 million new cases diagnosed in 2012. In recent years, in patients of breast cancer, prognostic value of preoperative CEA and CA15-3 levels has gained much attention. Study has revealed that preoperative plasma level of CEA combined with CA15-3 may provide useful information for diagnosis and treatment of breast cancer.

Aims & objectives: To correlate CA 15-3 and CEA level with clinicopathological parameters for early diagnosis and prognosis of metastatic breast cancer.

Materials and methods-This prospective study was done in department of biochemistry, IGIMS Patna. 75 diagnosed metastatic breast cancer patients of age group 30-70 years were included in this study. Correlation of CA15-3 and CEA levels with clinicopathological parameters were analysed.

Results: In 75 patients of metastatic breast cancer, elevated CA 15-3 and CEA levels were seen in 44 (58.6%) and 26(34.7%) patients, respectively. Level of CA 15-3 and CEA were significantly associated with different molecular subtypes ($p=0.025$ and $p=0.043$, respectively). CA15.3 level was more elevated in Luminal A, Luminal B, and HER2 positive cases whereas CEA level was more elevated in HER2 positive cases. Elevation of CA 15-3 was significantly more common in multiple site metastasis of breast cancer compared with a single site metastasis ($p<0.0001$). However, elevated CEA levels were not significant between patients with a single and those with multiple site metastasis.

Conclusions: Elevation of CA 15-3 and CEA levels were found to be significantly associated with different molecular subtypes of metastatic breast cancer and CA15-3 level was also significantly associated with multiple site metastasis of malignant breast cancer.

Keywords: metastatic breast cancer, cancer antigen 15-3 (CA15-3), carcinoembryonic antigen (CEA), clinicopathological parameters.

INTRODUCTION

Breast cancer is the most common cancer occurring in woman worldwide, nearly a quarter (25%) of all cancers with 1.67 million new cases arising in 2012. Women belonging to less developed regions have more number of cases compared to

more developed regions.⁽¹⁾ With advancement in early diagnosis and treatment, its mortality rate has declined. In case of treatment failure, survival of patients and quality of life is severely affected.⁽²⁾ Hence it is important to evaluate prognostic factors for taking more reliable decision during

treatment of breast cancer and to improve survival rate of patients. ⁽³⁾ Prognostic factors are clinicopathological parameters which includes tumor size, axillary lymph node status, hormone receptors status (estrogen receptors, progesterone receptors), human epidermal growth factor receptor 2(HER2). Using prognostic factors individually or combined can predict patient's outcome and treatment plan for better survival. ^(4,5)

More convenient prognostic markers of breast cancer are circulating level of tumor markers such as carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3), tissue polypeptide-specific antigen (TPS), cancer antigen 27.29 (CA27.29). ⁽⁶⁾ Among these carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) are most commonly recommended. ⁽⁷⁾ Carcinoembryonic antigen (CEA), a glycosylated cell surface glycoprotein found in normal cell and tumor cell but glycosylation differ in normal and tumor cells. CEA level usually increases in metastatic breast cancer. CA15-3, is also a glycoprotein belonging to mucin-1 (MUC-1) family, is over-expressed in tumor cells and altered glycosylation of CA15-3 makes it a useful tumor marker. ^(8,9)

In recent years, in patients of breast cancer, prognostic value of preoperative CEA and CA15-3 levels has gained much attention. Many Studies have revealed that preoperative plasma level of CEA combined with CA15-3 may provide useful information for diagnosis and treatment of breast cancer. However, this finding in breast cancer patients is controversial. ^(10,11) Previous reports have shown that preoperative levels of CEA and CA15-3 are related to clinicopathological parameters such as tumor size, axillary lymph node status, and hormone receptor status in patients of breast cancer. ^(12,13)

Breast cancer is a heterogeneous disease, and only histological classification does not satisfy variable clinical outcome of disease. Hence molecular classification is necessary to predict prognosis and outcome.

Breast cancer is classified as four molecular subtypes. Molecular subtypes classified as Luminal A (ER+and/or PR+, HER2-); Luminal B (ER+ and/or PR+, HER2+); HER2 positive (ER- and PR-, HER2+); and triple-negative (ER- and PR-,HER2-) tumor. ⁽¹⁴⁾

MATERIALS AND METHODS

This prospective study was done in the department of biochemistry, IGIMS, Patna. 75 diagnosed female patients of breast cancer in age group of 30-70 years visiting Surgical Oncology department were enrolled for the study. The proposal was approved by Institutional ethical committee. The study carried out between July 2017 to February 2019.

After overnight fasting, 5ml venous blood sample was collected from patients in plain vial. Patients' details regarding age, complete clinical details, general physical examinations were recorded in performa for each and every patient. Patients having male breast cancers, Carcinoma in situ, receiving Neoadjuvant chemotherapy, benign breast diseases and other major illness were excluded.

All the breast cancers patients were staged according to the American Joint Committee on Cancer (AJCC), TNM staging system. Distant metastasis refers to the presence of lesions at sites distant to the primary site. The sites of distant metastasis were as follows: lung, bone, liver, pleura.

Measurement of CEA and CA15-3 levels: 5ml venous blood was collected in plain vacutainer tubes. All samples were centrifuged at 4000 rpm. Serum was separated. Estimation of CA15.3 and CEA were performed with Chemimmunoassay analyser access2 (Beckman Coulter) in department of biochemistry, using calibrators, quality controls and reagent kit provided by Beckman Coulter. The upper limits of normal for CA 15-3 and CEA were 31 U/ml and 5 ng/ml, respectively.

Immunohistochemical evaluation:

The pathological specimens were reviewed by clinical pathologists.

Immunohistochemical staining was performed for ER, PR, HER2 evaluation. ER and PR positivity were defined by any positive nuclear staining $\geq 1\%$. HER2 was defined by IHC and graded between 0 and 3+. HER2-negative staining was defined as HER2 graded 0 or 1+; positive defined as 3+; HER2 scores of 2+ were HER2-negative or -positive according to the results of FISH (fluorescence *in situ* hybridization) analysis.

In this study breast cancer was classified as four molecular subtypes. Molecular subtypes classified as Luminal A (ER+and/or PR+, HER2-); Luminal B (ER+ and/or PR+, HER2+); HER2 positive (ER- and PR-, HER2+); and triple-negative (ER- and PR-,HER2-) tumor.

Statistical analysis

Statistical analyses were performed with the help of SPSS 22.0 software. The Chi-square test or the Fisher's exact test was used to compare the percentages of patients with elevated CA 15-3 and CEA levels with clinicopathological parameters. Similarly same statistical test were used to compare the percentages of patients with CA 15-3 and CEA elevation by the single site of metastasis or the multiple metastatic sites.

Statistical assessments were two-sided and level of significance was at $p < 0.05$.

RESULTS

The clinicopathological parameters of 75 metastatic breast cancer patients were summarized. The median age of patient was 44 years (range, 30-70 years).

Table 1 represents correlation between serum CA15-3 and CEA at initial diagnosis of metastatic breast cancer and clinicopathological parameters at the time of diagnosis. Elevation of serum CA15-3 and CEA were only significant in different molecular subtypes of breast cancer having p value 0.025 and 0.043 respectively. Other parameters like Age, histological grades were not significantly correlated with CA15-3 and CEA level. CA15-3 level elevated in molecular subtypes were as follows- LuminalA 66.67%(16/24), Luminal B 73.91%(17/23), HER2 Positive 62.5%(5/8), triple negative tumor 30%(6/20). CEA elevation were observed to be less than CA15-3 in molecular subtypes. CEA elevation in molecular subtypes are as follows- LuminalA 28.58% (8/28), LuminalB 13.04%(3/23), HER2 Positive 64.3%(9/14), triple negative tumor 37.5%(6/16).

Table 1. Correlation between serum CA15-3 and CEA at diagnosis of metastatic breast cancer and clinicopathological parameters

Characteristics	CA15-3			CEA		
	Normal (%) n=31	Elevated (%) n=44	p value	Normal (%) n=49	Elevated (%) n=26	p value
Age, years <35 ≥ 35	6(46.15) 25(40.32)	7(53.85) 37(59.68)	0.920	7(53.85) 42(67.74)	6(46.15) 20(32.26)	0.522
Histological grade III IV	10(43.47) 21(40.38)	13(56.53) 31(59.62)	1	11(55.00) 38(69.09)	9(45.00) 17(30.91)	0.389
Molecular subtypes Luminal A Luminal B HER2positive TN	8(33.33) 6(26.09) 3(37.50) 14(70.00)	16(66.67) 17(73.91) 5(62.50) 6(30.00)	0.025	20(71.42) 14(82.35) 5(35.71) 10(62.50)	8(28.58) 3(17.65) 9(64.29) 6(37.50)	0.043

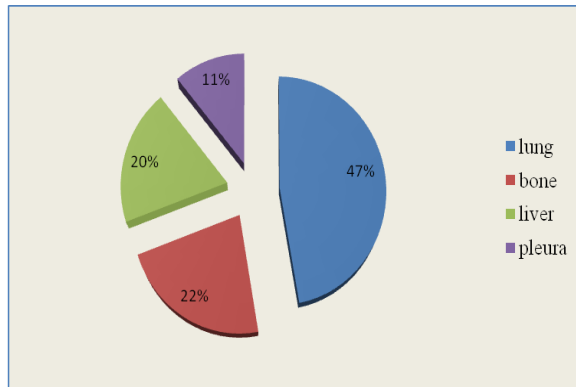
Number of Metastatic organ involved- A total of 36(48%) patients had a single organ metastasis and 39(52%) patients exhibited multiple organ metastasis as shown in table2. Patients with a single organ metastasis were distributed as follows: 17(47%) lung, 8(22%) bone, 7(20%) liver, 4(11%) pleura as mentioned in table 3.

Table 2. Single and Multiple organ involvement

Single organ involved	36(48%)
Multiple organ involved	39(52%)

Table 3. Number of patients with only one metastatic organ involved (n=36)

Lung	17
Bone	8
liver	7
Pleura	4



Correlation of CA15-3 and CEA with number of metastatic sites-

Among 36 patients with single metastasis, elevated level of CA 15-3 and CEA were identified in 11 (30.5%) and 15 (41.7%) patients, respectively. In multiple site metastasis, out of 39 patients elevated level of CA 15-3 and CEA were identified in 33(84.6%) and 11(28.2%) patients respectively as shown in table4.

Table 4. Level of CA15-3 & CEA with metastasis

Number of metastatic site	CA15-3			CEA		
	Normal (%) n=31	Elevated (%) n=44	p value	Normal (%) n=49	Elevated (%) n=26	p value
Single	25(69.44)	11(30.56)	<0.0001	21(58.33)	15(41.67)	0.327
Multiple	6(15.38)	33(84.62)		28(71.79)	11(28.21)	

DISCUSSION

Breast cancer is most common occurring malignancy that differs in their molecular and pathological characteristics. Despite advances in treatment, patients with early breast cancer develop relapse with distant metastatic disease. (15) To improve early detection of metastatic breast cancer CA 15-3 and CEA are widely used in the clinical setting. The utility of measuring CA 15-3 and CEA levels in breast cancer patients remains controversial. The European Group has recommended the use of CA15-3 and CEA for the prediction of prognosis and for the evaluation of recurrence and therapeutic effects. (16) However, according to ASCO guidelines, these markers should be used only for monitoring clinical parameters in the treatment of metastatic breast cancer. (17) The National Comprehensive Cancer Network (NCCN) guidelines do not recommend the use of CA15-3 and CEA as markers for clinical evaluation of breast cancer before initiation of treatment. (18)

It was previously shown that CA 15-3 and CEA levels were elevated in approximately 80% and 40% respectively, in patients diagnosed with metastatic breast cancer. (19,20) In the present study, elevated CA 15-3 and CEA levels at initial diagnosis were identified in 44 (58.66%) and 26

(34.67%) of the 75 patients, respectively, in accordance with previous findings.

Previous studies shows an association between elevated CA 15-3 levels and ER positivity, and reported that the incidence of increased CA 15-3 levels was higher in patients with ER-positive primary tumors compared to those with ER-negative tumors. (21,22) Our results indicated that CA 15-3 and CEA elevation at initial diagnosis of metastatic breast cancer were associated with breast cancer molecular subtypes (P=0.025 and P=0.043, respectively). Elevated CA 15-3 and CEA levels were most often observed in luminal subtypes, whereas the CA 15-3 and CEA elevation were lower in the HER2-enriched and TN subtypes. The reasons for these differences have not been fully elucidated. According to a previous study, the expression patterns of luminal, HER2-enriched and basal-like tumors are closely related with mature luminal, luminal progenitor and the basal stem/progenitor cells of normal breast tissue, respectively. (23) Based on findings, it is hypothesized that less differentiated subtypes lack certain circulating antigens. Luminal subtypes are specified by a high expression of hormone receptor (HR)-related genes, whereas HER2-enriched or basal-like subtypes exhibit a low expression of HR-related genes, indicating an association between

rise of CA 15-3 and CEA and HR expression.⁽²⁴⁾ The measurement of serum tumor markers is relatively easy as well as cost-effective. The incidence of the increase in CA 15-3 and CEA levels according to breast cancer molecular subtype may be useful in clinical practice.

Increase of CA 15-3 was significant in patients with ≥ 2 metastatic sites compared with a single metastasis, in agreement with previously reported findings. The significantly elevated CA 15-3 levels seen in cases with multiple metastases may reflect tumor burden.

CONCLUSION

Elevation of CA 15-3 and CEA level in metastatic breast cancer were found to be associated with breast cancer molecular subtype. These tumor markers are frequently increased in the luminal subtypes of metastatic breast cancer. In addition, an elevated CA 15-3 level was correlated with metastasis, whereas elevated CEA was observed regardless of metastatic site.

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