

## Diagnostic and prognostic role of CA 15-3 in breast carcinoma

Charmi R. Vyas<sup>1</sup>, Vijay C. Popat<sup>2\*</sup>, P. M. Santwani<sup>3</sup>, Suresh Bhanushali<sup>4</sup>, Deval Mehta<sup>5</sup><sup>1,5</sup>Tutor, <sup>2</sup>Associate professor, <sup>3</sup>Professor & Head, <sup>4</sup>Ex. Resident in pathology, M. P. Shah medical college, Jamnagar**ABSTRACT**

**BACKGROUND:** Breast cancer is one of the most common malignancy among women in most developed and developing regions of the world with nearly a million new cases each year. Prognosis of Breast cancer depends on evaluation of various parameters like tumor histologic grading, cell proliferation index, estrogen receptor status and lymph node status is of growing interest<sup>3</sup>. CA 15-3 mucin belonging to a large family of glycoprotein's encoded by the MUC1 gene. CA 15-3 determination is particularly useful in evaluating recurrence of disease and response to treatment. CA 15-3 is the most sensitive marker for the diagnosis of secondary breast cancer<sup>7</sup>. **MATERIAL AND METHODS:** For these purpose patients who were admitted in various wards like surgery and radiotherapy at G.G. Government hospital Jamnagar were considered. Total 100 cases studied. These included patients with suspected carcinoma of breast and presenting with lump in breast, pain, or discharge from nipple. Some cases presented directly with the metastatic disease and complaints of bone pain. These patients were investigated for CA 15-3 levels in their serum with the use of ELISA (Enzyme Linked Immunosorbant Assay) test. Both Pre operative and post operative values were measured. post operative values were measured in few cases to see the changes in CA 15-3 value after tumor resection. **OBSERVATION AND RESULT:** Marker levels were correlated with the histopathological findings like size, grade etc. pre and post operative values were measured for prognostic purpose, to monitor response to treatment and to detect recurrence and metastasis. Detailed history, clinical examination, investigation, gross findings, microscopic cytological and histopathological findings were evaluated and following observations and results were found. In present study it was observed that majority of patients belonged to age group of 41-60 yrs, In majority of patients size of lesion was between 2-5cm and grade was 2. It was found that mean pre op and post op CA 15-3 was higher in metastatic and recurrent cases than other cases. It was found that post treatment CA 15-3 is 83.3% sensitive and 98.1% specific in detection of recurrent or metastatic diseases. **CONCLUSIONS:** CA 15-3 is one of the first circulating prognostic factors for breast cancer. Preoperative concentrations thus might be combined with existing prognostic factors for predicting outcome in patients with newly diagnosed breast cancer. At present, the most important clinical application of CA 15-3 is in monitoring therapy in patients with advanced breast cancer that is not assessable by existing clinical or radiologic procedures.

**Key words:** CA 15-3, serum tumor marker, breast carcinoma, prognosis

**INTRODUCTION**

Breast cancer is one of the most common malignancy among women in most developed and developing regions of the world with nearly a million new cases each year. It accounts for nearly 21% of all cancers among women worldwide.<sup>1</sup> In India, breast cancer is the second most common cancer (after cervical cancer) with an estimated 1,15,251 new diagnoses and the second most common cause of cancer-related deaths with 53,592 breast

cancer deaths in 2008<sup>2</sup>. Early diagnosis is primary aim. Prognosis of Breast cancer depends on evaluation of various parameters like tumor histologic grading, cell proliferation index, estrogen receptor status and lymph node status is of growing interest<sup>3</sup>. Histologic grade has been an important prognostic indicator that can predict overall and metastasis free survival for local and regionalized breast cancer<sup>4</sup>. Cytological grading has shown a positive correlation with histologic grade, therefore cyto grade is useful in predicting histo grade preoperatively. Cytological grade would thus provide relevant information on the tumor biological behavior and could be a useful parameter to take into consideration when selecting neo-adjuvant therapy<sup>5</sup>. Now a days, immunohistochemistry and serum tumor

**\*Corresponding Author:**

Dr. Vijay Popat  
A-3, Rutviraj apartment,  
Opp. Essar House,  
Jamnagar- 361 008  
Email-Id: drvijaypopat@yahoo.co.in  
Contact No: 919825076855

marker detection is widely used in evaluation in case of breast carcinoma. Tumor markers may be used in diagnostic, prognostic evaluation and follow up<sup>6</sup>. CA 15-3 mucin belonging to a large family of glycoprotein's encoded by the MUC1 gene. CA 15-3 determination is useful in diagnosis and prognosis of breast cancer. With the emphasis that it has significant role in evaluating recurrence of disease and response to treatment. CA 15-3 is the most sensitive marker for the diagnosis of secondary breast cancer<sup>7</sup>.

**AIMS AND OBJECTIVES**

- To study the diagnostic and prognostic role of serum tumor marker CA 15-3 in breast carcinoma patients.
- To establish correlation between values of serum CA 15-3 level and cytopathological diagnosis.
- To monitor the response to treatment in women diagnosed with breast carcinoma.
- To analyze correlation of serum CA 15-3 level with clinico-pathological and histo-pathological parameters like age, size of lesion, grade of tumor, no. of lymph nodes involved and stage of disease.
- To detect the early recurrence of disease and metastatic breast carcinoma diseases with CA 15-3 level.

**MATERIALS AND METHODS**

The study was carried out at M.P. Shah medical college and Guru Govindsingh Government hospital of Jamnagar during April 2010 to April 2012. Total 100 patients of known case of carcinoma breast were selected. All patients were females and diagnosis of breast carcinoma were done by cytology and/or histopathology. Emphasis was also given on supportive investigations like radiological investigation i.e. ultrasonography, mammography findings and blood investigations. These patients were investigated for CA 15-3 levels in their serum with the use of ELISA (Enzyme Linked Immunosorbant Assay) test. The ELISA test was carried out by DIAMETRA CA 15-3 assay. It is a quantitative assay done with solid phase enzyme immobilized on microwell plates,

the other conjugated with horseshoepoxidase(HRP) after incubation, the separation bound-free is obtained with simple solid phase washing. In all patients preoperative CA 15-3 values were measured while in 60 cases CA 15-3 values were measured after tumor resection to see the change in value. Post operative values were measured after 7 days of surgical resection. The enzyme in the bound fraction reacts with the substrate(H<sub>2</sub>O<sub>2</sub>) and the TMB substrate and develops a blue colour that changes into yellow when stop solution (H<sub>2</sub>SO<sub>4</sub>) is added. The colour intensity is proportional to CA 15-3 concentration in the sample. CA 15-3 concentration in the sample is calculated using a standard curve.

**Reference value:** Healthy women are expected to have CA15-3 value below 35IU/ML CA 15-3 values were correlated with cyto-pathological and/or histo-pathological diagnoses of mass.

**OBSERVATION AND RESULTS**

**Table1: Information Regarding Studied Cases**

Sr. No.	Studied Cases	No.
1	Total cases studied	100
2	Pre-Tx CA 15-3 value measured	100
3	Post-Tx CA 15-3 value measured	60
4	Duration of Post-Tx value measured after surgical removal	7 days
5	Duration of Post-Tx measurement for pts taking radio/chemotherapy	3 cycles
6	Post op values not measured	40

Total – 100 cases were studied. These cases were patients admitted to various departments of our hospital. These included patients with carcinoma of breast and presenting with lump in breast, pain, or discharge from nipple. Some cases presented directly with the metastatic disease and complaints of bone pain. Pre-operative CA 15-3 value measured of every patient before surgery or before starting neoadjuvant therapy i.e radiotherapy or chemotherapy. Marker levels were correlated with the histopathological findings like size, grade, lymph node status and stage of tumor. Pre and post operative values of CA 15-3 were measured for prognostic purpose, to monitor response to treatment as well as to detect recurrence or metastasis. Post operative values were measured after 7

days post operatively or after 3 cycles of radiotherapy or chemotherapy. Detailed history, clinical examination, investigation, gross findings, microscopic cytological and histopathological findings were evaluated and following observations and results were found.

**Table 2: Type Of Sample**

TYPE OF SAMPLE	NUMBER OF PATIENTS
Biopsy	26
Biopsy f/b MRM	24
FNAC f/b MRM	50

In 50% of cases diagnosis of carcinoma breast was confirmed with FNAC following which MRM was done. In rest 50% cases biopsy of breast lump, either excisional or incisional type was done out of which in only 24% of cases biopsy was followed by MRM.

**Table 3: Age Distribution Of Cases Of Carcinoma Breast With Mean Ca 15-3 Value**

Age	No. of cases	Mean Serum CA 15-3 Levels(U/ml)
20-40 yrs	15	40.108
41-60 yrs	53	55.62
More than 60 yrs	32	66.55
Total	100	

**Table 4: Positivity Rate Of Ca 15-3 In Patients According To Age**

Age	No. of cases	No. of patients whose CA 15-3 value is more than 35	Positivity rate (%)
21-40 yrs	15	6	40
41-60yrs	53	35	66.03
More than 60 yrs	32	27	84.37

In present study it was observed that majority of patients belonged to age group of 41-60 yrs. Mean value of CA 15-3 level was highest in patient of more than 60 years of age. Maximum no. of patients i.e 84.37% among lesion larger than 5 cm showed serum CA 15-3 levels more than 35 unit/ml. Thus, mean CA 15-3 level as well as positivity rates( i.e cases showing serum CA 15-3 value more than 35 U/ml ) increased as age of the carcinoma breast patient increased.

**Table 5: Size Distribution Of Cases Of Carcinoma Breast With Mean Ca 15-3 Value.**

Size (cm)	No. of patient	Mean serum CA 15-3 levels ( U/ml)
Less than 2	8	33.2
2-5	63	53.14
More than 5	29	71.22
Total	100	

**Table 6: Positivity Rate Of Ca 15-3 In Patients According To Size**

Size	No. of patients (n=100)	No. of patients whose CA 15-3 value is more than 35U/ml (positivity rate)	Positivity rate %
Less than 2	8	2	25
2-5	63	42	66.66
more	29	24	82.75

In present study, it was observed that in majority of patients, size of the lesions was between 2-5 cm. Mean value of CA 15-3 level was highest i.e 71.22 U/ml in patients with lesion larger than 5cm. Maximum number of patients among lesion larger than 5 cm showed serum CA 15-3 levels more than 35 U/ml. Thus, mean CA 15-3 level as well as positivity rates increased as size of the lesion increased in carcinoma breast patients.

**Table 7: Distribution Of Cases Of Carcinoma Breast According To Grade With Mean Ca 15-3 Value**

Grading	No. of patients	Mean serum CA 15-3 levels (U/ml)
1	21	32.13
2	56	55.81
3	23	81.68

**Table 8: Positivity Rate Of Ca 15-3 In Patients According To Grade Of Tumor**

Grading	No. of patients	No. patients whose CA 15-3 value is more than 35	Positivity rate (%)
1	21	3	14.28
2	56	45	80.35
3	23	20	86.95

In present study it was observed that majority of cases of carcinoma breast belonged to nuclear grade 2. Mean serum CA 15-3 values were highest i.e 81.68 U/ml in grade 3 patients. More number of cases of nuclear grade 3 showed higher CA 15-3 values compared to cases of grade 1 and 2. Thus, mean serum CA 15-3 value as well as positivity rates increased with nuclear grade.

**Table 9: Distribution of Cases According To No. Of Lymph Nodes Involved**

Number of lymph nodes	Number of patients	Percentage
Less than 4	32	43.2%
More than 4	42	56.75%
Total	74	

**Table 10: Mean Ca 15-3 Level According To No. Of Lymph Nodes Involved**

Number of lymph nodes	Mean serum CA 15-3 levels (U/ml)
Less than 4	46.12
More than 4	72.48

**Table 11: Positivity Rate Of Ca 15-3 In Patients Accordiing To Number Of Lymph Nodes Involved**

Number of lymph nodes	Number of patients	Number of patients whose CA 15-3 value is more than 35 U/ml	Positivity rates(%)
Less than 4	32	18	56.2
More than 4	42	38	90.47

In present study, cases undergone MRM were divided according into cases with less than 4 lymph nodes involved and cases with more than lymph nodes involved. It was found around 56.7% of cases showed more than 4 lymph nodes involved. Mean serum CA 15-3 level of patients with more than 4 lymph node involvement was 72.48U/ml and 90.47% of cases with more than 4 lymph node involved showed CA 15-3 levels more than 35U/ml. Thus, Mean serum CA 15-3 levels and positivity rate of CA 15-3 increased as number of lymph node involvement increased in carcinoma breast patients.

**Table 12: Comparison Of Mean Ca 15-3 Values , Preop And Post Op Cases**

Cases	Number	Mean serum CA 15-3 values (U/ml)
Pre operative cases	60	69.8
Post treatment cases	60	36.85

In all the 100 cases in present study preoperative CA 15-3 levels were measured. Post treatment values were measured in 60 cases following MRM or chemotherapy. In rest of cases postoperative value detection could not be done as some of the patients MRM was done in our hospital and few patients did not come for the follow up.

- It was found that 54 cases .i.e., 90% of cases showed decreased value of CA 15-3 post operatively.
- The response to treatment either the surgery or the chemotherapy was seen in the form of decreased value of mean CA 15-3 levels. This decrease in CA 15-3 value directly correlated with tumor removal or decrease in tumor size.

**Table 13: Comparison Of Mean Ca 15-3 Values In Metastatic, Recurrent And Other Cases**

	Mean preop CA 15-3 values	Mean post op CA 15-3 values
Metastatic cases	101.7	89.75
Recurrence cases	107.3	105.6

Other than metastatic or recurrence cases	53.80	30.38
---	-------	-------

Mean pre op as well as post op CA 15-3 was higher in these metastatic and recurrent cases than other cases.

**Table 14: Statistical Study**

Disease Test	Metastatic/recurrent disease present	Metastatic/recurrent disease absent	Total
	High CA 15-3 VALUES	05	
LOW CA 15-3 VALUES	01	53	54
TOTAL	06	54	60

In present study the role of CA 15-3 in detection of metastatic disease or recurrent disease was evaluated and observed that post treatment CA 15-3 is 83.3% sensitive and 98.1% specific in detection of recurrent or metastatic disease post treatment. Similarly positive predictive value of CA 15-3 was and negative predictive value was found to be 83.5 and 98.1%.

- Pre-operative and post operative CA 15 -3 values were compared “using paired t test.”

**Table 15: Statistical Study**

CA 15-3 LEVEL	MEAN	N	STD. DEVIATION	T TEST	P VALUE
Pre op.	69.8	60	20.74	8.095	<0.0001
Post op	36.85	60	23.86		

Here p value is highly significant. As p value <0.0001

With above value t value found was = 8.095

- These findings suggest that CA 15-3 has definitive prognostic role (p value <0.0001) in breast carcinoma. Even with normal preoperative CA 15-3 values, post operative values are important to detect any recurrence or metastasis. And pre operative value is important for post operative follow up patients.

**DISCUSSION**

About 30 years before the writing of this study began, the first author was given the opportunity to begin a series of experiments that would lead to the discovery of CA 15-3 as a human tumor marker, and to the myriad avenues of CA 15-3 research that are being actively pursued at the present moment. In case of malignant tumors, recurrence or metastasis

remains a major problem. So, some cost effective investigation is required to detect recurrence at earlier stage when it is still resectable or manageable. It is equally proportional to size of tumor and higher cyto-pathological and histo-pathological grade at the same time it reflects recurrence and metastasis by significant rise in its serum value. It is valuable diagnostic and prognostic tool in developing country like India. Cost effectiveness and easy availability of this diagnostic modality is really useful at PHC and even rural areas for patients. One of the tests to diagnose carcinoma is use of tumor markers. ELISA test to measure CA 15-3 is reliable, sensitive, rapid and cost effective test. CA 15-3 is the preferred tumor marker of choice for detection of tumor recurrence after surgery or radiotherapy in cases of carcinoma breast with pretreatment elevated CA 15-3. It is also a good serum marker for monitoring response to treatment for carcinoma breast patients.

### CONCLUSION

- CA 15-3 values in normal female is <35 U/ml. Values >35U/ml are considered abnormal. CA 15-3 value although has definitive diagnostic value, it cannot be used as a sole diagnostic measure and supportive investigations like Radiological diagnosis, FNAC and/or histo-pathological study should be used for diagnosis.
- Higher the grade or stage of the tumor the positivity of rate of tumor marker also increases.
- Our data in patients with breast cancer suggest that elevated preoperative CA 15-3 levels are associated with advanced TNM staging, histological grade and thus higher risk of recurrence locally or at distant sites.
- Elevated serum level of CA 15-3 correlated with increasing age, size and lymph node status.
- The sensitivity of CA 15-3 increases to 95% in patients with advanced disease.
- ELISA is rapid, sensitive, and cost effective test for measurement of cancer antigen 15-3.

- Pre and post operative CA 15-3 values are important to study, decrease in CA 15-3 value after surgery, radiotherapy or chemotherapy correlates with efficiency of treatment.
  - Post operative CA 15-3 value has definitive prognostic value to detect any metastasis or recurrence after successful surgery or chemotherapy.
  - Chances of recurrence are more with tumors with higher pre operative value.
- Thus, CA 15-3 is an important prognostic indicator, good predictor for relapse and important for therapeutic monitoring in breast cancer patients.

### REFERENCES

1. Parkin DM, Pisani P, Ferlay J. Estimates of the world wide incidence of 25 major cancers in 1990. *Int J Cancer*. 1999 Mar; 80(6):827-841.
2. Ferlay J BF, Pisani DM. Cancer Incidence, Mortality and Prevalance Worldwide. *Globocan 2000*, version 1.0.2001.
3. Taniguchi E, Yang W, Nakamura Y, Shan L, Nakamura M, et al. Cytological grading of invasive breast carcinoma. Correlation with clinicopathologic variables and predictive value of nodal metastasis. *ACTA cytol*. 2000;44(4):587-591.
4. Howell LP, Gandour-Edwards R, O'Sullivan D. Application of the Scaeff-Bloom-Richardson tumor grading system to Fine Needle Aspirates of breast. *Am J Clin Pathol* 1994;101:262-265.
5. Robles-Frias A, Gonzalez- Campora R, Martinez-Parra D, Robles-Frias M, Vazquez-Cerezuda T, Otal-Salaverr C, et al. Robinson cytologic grading of invasive ductal breast carcinoma. Correlation with histologic grading and regional lymph node metastasis. *Acta Cytol*. 2005;49(2):149-153.
6. [www.asco.pubs.org](http://www.asco.pubs.org).
7. Aziz DC, ET al Speciality Laboratories,(1991)
8. Aziz DC. *A J Clinical Pathology* 5:422-38(1992)
9. Aziz DC, et al *J Clinical Pathology* 98:105-11(1992)

10. Clark GM, et al N Engl J Med 320:627-633(1989)
11. Elledge RM, et al Annu Rev Med 44:201-10(1993)
12. Foekens JA, et al Cancer Res 50-3832-7(1990)
13. Isola J, et al Cell Biochem (Suppl 16D): 101(1992)
14. Antonella Daniele, Rosa Divella, Paolo Trerotoli, Maria Elena Caringella, Angelo Paradiso, Porzia Casamassima, Ines Abbate, Michele Quaranta and Antonio Mazzocca : Clinical Usefulness of Cancer Antigen 15-3 in Breast Cancer Patients Before and After Surgery, The Open Breast Cancer Journal, 2013, volume 5,1-6.
15. M. Gion, R. Mionel, Nascimben, M. Valsecchil, C. Gatti, A. Leon & G. Brusca : The tumor associated antigen CA15.3 in primary breast cancer. Evaluation of 667 cases, Br. J. Cancer (1991),63,809-813.
16. Eyad Fawzi Alsaeed, Huda Abdulkarim, Mutahir A Tunio : Elevated preoperative serum cancer antigen 15.3 levels are associated with reduced disease-free survival : a single – institution experience. Dove press journal, Breast cancer – targets and therapy, july 2013.
17. Dhafir Al-azawi, Gabrielle Kelly, Eddie Myers, Enda W McDermott, Arnold DK Hill, Michael J Duffy and Niall O Higgins : CA 15-3 is predictive of response and disease recurrence following treatment in locally advanced breast cancer. BMC Cancer 2006,6:220.
18. S. Velaiutham, Nur Aishah Taib, KL Ng, BK Yoong, Cheng Har Yip : Does the Pre-operative Value of Serum CA15-3 Correlate with Survival in Breast Cancer ? Asian Pacific Journal of Cancer Prevention, Vol 9.2008,445-448.