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SERIAL ESTIMATION OF CA 15.3 CAN PREDICT THE OUTCOME IN PATIENTS WITH BREAST CANCER – A PROSPECTIVE STUDY.

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ABSTRACT

CA 15.3 is widely applied for long term follow-up and monitoring therapy of breast cancer patients. The present study was conducted to assess the role of CA 15.3 in association with Nottingham Prognostic Index (NPI) to detect outcomes of breast cancer patients. Blood samples were collected from 85 nonmetastatic breast cancer patients and 57 patients with benign breast disease (BBD). CA 15.3 was measured from serum by ELISA. By comparing pre and post op CA 15.3 level, a significant decline in post op values were observed in majority of patients. NPI scores of patients were calculated. 18-24% of patients who had persistently elevated CA 15.3 in post-op follow-up and scored NPI>5.4, showed PO in term of recurrence/relapse/distant metastasis. Also PO (NPI>5.4) was correlated with large TS, different grades and with nodal metastasis. Raised post-op CA 15.3 along with high NPI can be predictive to derive outcome in breast cancer patients.

KEYWORDS: **Primary breast cancer, CA 15.3, Nottingham Prognostic Index, outcome**

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INTRODUCTION

Breast cancer is one of the most common malignancies among the women worldwide[1]. In spite of advances in the therapeutics modalities for the management of breast cancer, survival depends on biological behavior of the disease. Till now, traditional tumour markers like tumour size (TS), axillary lymph node status and histological grading[2] have been used as a prognostic tool. These prognostic parameters cannot predict the risk of recurrence (rec) or relapse (rel) or distant metastasis (dm). Carcinoma associated antigen 15.3 (CA 15.3) is one such marker. It is easily accessible from blood serum and can be measured pre & post-operatively. Furthermore, it is cost-effective, reproducible, specific and sensitive[3]. Its main application is for detection of disease recurrence after primary treatment in cancer, for monitoring therapy in advanced disease and for the surveillance of breast cancer patients[4]. Study reveals that CA 15.3 is encoded by MUC1 gene located on chromosome 1q. MUC1 apoprotein is a large transmembrane protein and it is aberrantly glycosylated in many type of cancer[5,6]. It has been reported that this cancer antigen is over expressed in >90% of breast carcinomas and metastasis[7,8]. Nottingham prognostic index (NPI) is a parameter which can be used to determine the need for adjuvant therapy in patients. The index is calculated by a formula consisting of TS, tumour grade and number of metastatic lymph node[9]. In NPI, grade and lymph node stage has equal weighting. Patients having low NPI show much better prognosis than those having high NPI [10]. This work is based on the hypothesis that the pre-op level of CA 15.3 was reflective of the total tumour burden in a patient's body. The magnitude should decline below the cutoff point after removal of the locoregional tumor load. In patients whose CA 15.3 level remain persistently elevated or in whom the value decreased but remain above normal cut off without any detectable metastasis, the occult systemic tumour load is hypothesized to be high. In them early systemic recurrence is expected. If the value increases during follow-up, it would be assumed that a secondary source has evolved due to recurrence or distant metastasis. Hence it is important to identify those patients who will have aggressive biological disease and poor outcome (PO) in the form of rec/rel/dm. These high risk patients are supposed to benefit most from adjuvant chemotherapy. In the present prospective study, we investigated the outcome of Indian breast cancer patients by evaluating the relationship of elevated post-op CA 15.3 level with NPI and other histological parameters like TS, grades and nodal metastasis.

MATERIALS & METHODS

(i) Selection of patients

142 female patients attending at Breast Service, Dept. of General Surgery, IPGME&R/SSKM Hospital, Kolkata from Jan 2009 to Dec 2010 were studied. 85 patients who had non metastatic breast cancer were included in study arm. 57 patients with benign breast disease (BBD) were included as control. Patients with any other malignancy from their previous history or having distant metastasis (revealed in staging investigations at the time of diagnosis) were excluded from the study.

(ii) Sample collection and assay procedure

Blood samples were collected in clotting vials from all patients and serum was separated by centrifugation. CA 15.3 level was measured on pre-op day and repeated on 7^{th}, 30^{th},180^{th},360^{th},560^{th} and 720^{th} post-op day by sandwiched method of ELISA. Assay procedure was followed according to Accudiag ELISA kit (Ref 6333Z; LOT 112031903). Optical density was read at 450nm.

(iii) Calculation of results

Concentration of CA 15.3 was determined from the standard curve taking the mean absorbance of each specimen. A cut off value for the level of CA 15.3 was fixed at 25U/ml [11].

(iv) Treatment and follow-up of patients

Patients were treated with either modified radical mastectomy (MRM) or breast conservation therapy. After completion of surgery, radiotherapy and appropriate adjuvant chemotherapy or hormone therapy was administered as per international guidelines.

Clinical follow-up such as history taking, physical examination and lab tests including liver function tests, complete blood count, chest radiography, abdominal and breast USG ,mammography was used as for detection of local or distant relapse.

(v) Calculation of NPI

NPI was calculated by the formula T S $\times$ 0.2 + Nodal status + Grade.

Where no node refers to nodal status 1, 1-3 nodes refers to 2, more than 3 nodes refers to 3 and grade I equal to 1, grade II equal to 2, grade III equal to 3 was taken[10]. To monitor prognosis, cut-off point was set at NPI≤5.4[12].

(vi) Ethics

Before commencement of project, human ethical committee clearance was obtained for carrying out the project work. The work adhered to the principles expressed in the Declaration of Helsinki. Written informed consent form in three languages (English, Hindi & Bengali) was obtained from each of the patients prior to their recruitment in the study.

(vii) Statistical analysis

Statistical Analysis was performed with Epi Info (TM) 3.5.3. Descriptive statistical analysis was performed to calculate the means with corresponding standard errors (s.e.). Test of proportion was used to find the Standard Normal Deviate (Z) to compare the different proportions. T-test was used to compare the means. Significance level was set at $p<0.05$.

RESULTS

Table 1

Comparison of pre and post operative CA 15.3 in patients with breast cancer

Time interval	CA 15.3<25 u/ml	CA 15.3>25 u/ml	p value*
Pre-op	10	75	<0.01
7th post-op day	69	16	<0.01
30th post-op day	67	18	<0.01
6th post-op month	68	17	<0.01
12th post-op month	65	20	<0.01
18th post-op month	64	21	<0.01

**** Significance of proportion of patients with CA 15.3<25 U/ml with respect to proportion of patients with CA 15.3>25 U/ml.***

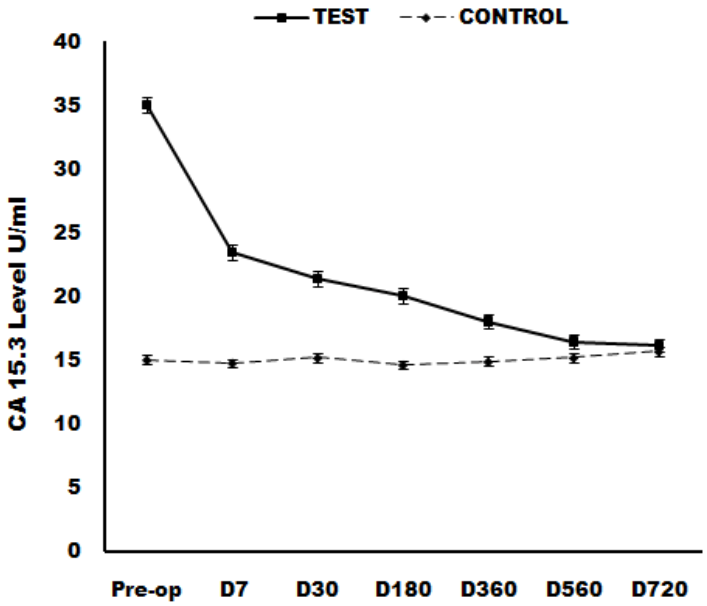

Figure 1

CA 15.3 level in Control and Test. Mean pre-op CA 15.3 of Control was 15.04 U/ml which was almost static in follow-up days. In Test group, pre-op level (35.04 U/ml) reached below the cutoff point on the next post-op days.

To monitor the change of the marker level in the post-operative period, serum CA 15.3 was measured on pre-op day and 7th, 30th, 180th, 360th, 560th and 720th post –op days. The patients with BBD showed average pre-op CA 15.3 level of 15.04 U/ml. The mean CA 15.3 value was almost static in the follow-up. In breast cancer group, the mean pre-op CA 15.3 level was very high (mean 35.04 U/ml) compared to control group. Immediately on the 7th post-op day, the mean marker level decline to 23.48 U/ml (Fig 1). On further follow-up, proportion of patients with CA 15.3<25 U/ml was significantly higher than proportion of patients with CA 15.3>25 U/ml ($p<0.01$) (Table 1). It was observed that 18-24% of patients had persistently raised CA 15.3 level in the follow-up period.

Table 2

Outcome of breast cancer patients according to NPI score

N.P.I.	N	Post-op CA 15.3<25 U/ml	Post-op CA 15.3>25 U/ml	Poor outcomes	p value†
≤ 5.4	57	1	1	2	NS
> 5.4	28	0	19	19	0.01

†***Significance of PO with respect to NPI score; NS: Not significant***

In table 2, breast cancer patients had been stratified into two groups on the basis of NPI score. Patients with NPI≤5.4 (n=57) and patients with NPI>5.4 (n=28). 19 PO was found in terms of local rec or dm in the patients with NPI>5.4 and CA 15.3>25 U/ml (Table 2). Test of proportion showed that proportion of PO had been observed significantly higher in patients with NPI>5.4 than patients with NPI ≤ 5.4 ($p<0.01$).

Table 3
CA 15.3 level of control group and different sub groups of breast cancer patients

Variable	N	CA 15.3 Mean ± SEM u/ml	CA 15.3 Median	Significance Vs benign††	CA 15.3<25 u/ml	CA 15.3>25 u/ml	Poor Out comes	p value§
Benign Breast Disease Group	57	15.04 ± 0.33	14.8	–	57	0	–	–
Breast Cancer Sub-Group								
Tumor size (cm)								
<2	24	33.00 ± 1.45	33.95	<0.01	3	21	3	NS
2 to 5	48	36.12 ± 0.77	37.3	<0.01	2	46	6	NS
>5	13	34.83 ± 1.72	35.2	<0.01	1	12	12	S
Grade								
1	22	31.30 ± 1.33	30.15	<0.01	2	20	0	NS
2	42	35.75 ± 0.91	36.5	<0.01	3	39	11	S
3	21	37.54 ± 1.03	39.5	<0.01	1	20	10	S
No. of nodal metastasis								
0	25	32.27 ± 1.27	33.6	<0.01	3	22	0	NS
1 to 3	24	34.86 ± 1.25	36.75	<0.01	2	22	2	S
4 to 9	20	37.55 ± 1.14	38.5	<0.01	1	19	9	S
>9	16	36.51 ± 1.40	38.7	<0.01	0	16	10	S

†† Significance of mean pre-op CA 15.3 level with respect to that of benign breast disease group.
§Significance of PO with respect to different TS, grades and nodal metastasis. Proportion of patients with PO was significantly higher for TS>5 cm (p<0.01) compared to TS <2 cm and TS =2-5 cm, all three grades and with nodal metastasis. No significant difference was found between the proportion of patients with PO for TS <2 cm and TS =2-5 cm.
NS: Not significant

As per Table 3, 57 patients were represented with BBD group and breast cancer group was subdivided according to varying TS, grades and nodal metastasis. Mean CA 15.3 of each breast cancer subgroups was found to be significantly high ($p<0.01$) than that of BBD group. In TS subgroup, it was found that 21 patients out of 24 with TS less than 2 cm had elevated CA 15.3 level and among them 3 showed PO in the follow-up. Similarly 6 patients with TS between 2-5 cm and 12 patients with TS>5 cm from CA 15.3>25 U/ml group showed PO (NPI>5.4). Incidence of PO occurs from grade 2 and grade 3. In case of lymph node metastasis, PO (NPI>5.4) was found in patients only with metastasis. 2, 9, and 10 PO (NPI>5.4) was obtained from nodal metastasis group 1-3, 4-9 and >9 respectively. All of them had post-op CA 15.3 level>25 U/ml. From test of proportion, it was obtained that proportion of patients with PO was significantly higher for TS>5 cm ($p<0.01$) compared to TS <2 cm and TS =2-5 cm, for grade 2 & 3 compared to grade 1 ($p<0.05$) and for increasing number of nodal metastasis ($p<0.01$-0.05) (Table 3)

DISCUSSION

Measurement of serum CA 15.3 level is a good tool in predicting prognosis, observing treatment response and in the long term follow up of breast cancer patients[3]. In the present study, the hypothesis was that the pre-op level of CA 15.3 was reflective of the total tumour burden in an individual body. It was expected that the value of this protein was likely to decline after removal of the locoregional tumour load. We found that the pre-op patients with tumours exhibited significantly high CA 15.3 value compared to control. Following surgery, immediately on 7th post-op day, the mean value declined to cut off level. This indicates that the primary source of production of excess CA 15.3 was removed. The

result was in agreement with previous study reported by Ebeling F.G. et al., in 2002 where they compared pre and post-operative values of CA 15.3 and found that the marker level had decline in post-surgery[2]. At the end of our study period, though the mean CA 15.3 level of the majority was 16.18 U/ml but some patients (18-24%) had remained above normal cut off level and they showed PO as well. This result was supported by the study of Martin A et al[13] who reported that in a univariate analysis, elevated CA15.3 levels were significantly associated with a lower probability of both relapse-free and overall survival in the overall group of patients. PO of breast cancer patients was correlated with NPI. By analyzing individual NPI score, it was found only 2 PO with NPI≤5.4. Significant PO was observed in high NPI score. These patients also had persistently elevated CA 15.3. Possibly, high serum CA 15.3 level was due to release of tumour associated antigen from occult metastasis in systemic circulation. This was indicator of vascularisation of tumor and poor prognosis. In 2001, D' Eredita et al[14] demonstrated the prognostic value of NPI. They reported NPI score< 3.4 as Good, NPI score> 3.4 and = 5.4 as Moderate and NPI score> 5.4 as Poor.

Mean pre-op CA 15.3 level between BBD and breast cancer subgroup (patients having different TS, histological grades and lymph node metastasis) was compared. It was observed that the mean pre-op value of BBD patients was significantly less ($p<0.01$) from that of patients possessing tumour. This was consistent with the fact that since the patients with BBD had no occult tumour; their marker level was below the cutoff point. O'Hanlon et al. (1995)[15] reported that there was significant difference in CA 15.3 levels between BBD and stage I, II, III and IV of primary breast cancer. Pons Anicet DMF et al. (1987)[16] published a study where they showed that 16% of patients with localized breast cancer had CA 15.3> 25 U/ml and the levels increased with tumour size. Next it was observed whether there was any correlation between PO (NPI>5.4) and increasing TS, different grades and with or without nodal metastasis. The incidence of PO was observed in patients with CA 15.3>25 U/ml for all TS subgroup. Significantly higher proportion of PO were observed in patients with CA 15.3>25 U/ml for TS >5 cm compared to TS <2 cm and 2-5 cm. No PO was observed in patients having no nodal metastasis. As with increasing number of nodal metastasis, number of PO increased significantly. We obtained 47% and 62% PO (NPI>5.4) in the patients who had persistently high CA 15.3 U/ml in the post–op days and nodal metastasis 4-9 and >9 respectively. In 2012, W.D. Foulkes[17] reported that tumor size and axillary lymph node status are independent factors for the measurement of outcomes. Women with 10-20 mm primary tumor size and 4 or more metastatic lymph node had better 5 year survival than those having larger (20-30 mm) tumor size but same number of lymph nodes involved by cancer. Another study suggested that a larger tumour had correlated with more positive lymph nodes, thus their interaction further influenced the survival from breast cancer [18]. Occurrence of PO was also found across all three grades. 28% PO from grade 2 and 50% PO from grade 3 were found. Though significantly high proportion of PO was found in case of large tumour (>5 cm), node positive group and high tumour grades (2 & 3).

CONCLUSION

High risk patients can be screened by the serial measurement of post-op CA 15.3 level. The group of breast cancer patients with persistently elevated post-op CA 15.3 and high NPI (>5.4) can have bad outcomes. Other traditional prognostic markers like large tumor size, high grade and node positivity had showed correlation with bad outcomes. It has got a statistically significant role. However to establish it as gold standard further study with long term survival data is to be contemplated.

CONFLICT OF INTEREST

Conflict of interest declared none.

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