Original Research Article

A Study to Evaluate the Effect of Neo-adjuvant Chemotherapy on Hormonal and Her-2 Receptor Status in Carcinoma Breast

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Abstract

Background: The advances in diagnosis and treatment, in the management of breast cancer have led to excellent cure rates for tumors detected in early stage. Even patients with stage III disease have 5 years survival rates in the range of 50-70%. The search for predictive and prognostic factors in breast cancer represents a major challenge. It is important to distinguish prognostic factors from predictive factors.

Aim: The aim of the surgery was to compare the immunohistochemical expression of estrogen, progesterone and Her-2 receptor status in breast cancer before and after neo-adjuvant chemotherapy.

Materials and methods: In this study, the total of 50 cases of locally advanced breast cancers was included. Cases of carcinoma breast requiring preoperative chemotherapy from January 2015 to January 2017 were recruited in to the study after informed consent.

Results: In this study, the total of 50 cases of locally advanced breast cancers was included. Among them most of the cases belonged to the age group of 40 to 60. The extremes of ages (<30 and >60) comprised of only 14 % of the cases. In total of 50 cases 32 patients were pre-menopausal which was accounting for 64% of cases, and remaining patients were post-menopausal. Out of 20 cases of triple negative in this study complete response was seen in 6 cases accounting for about 30%. In our study there were a total of 24 changes in the receptor status post chemotherapy altogether out of which 11 changes were seen in Her2neu group. Changes in the ER and PR group accounted for 8 and 5 cases respectively. Out of 14 cases of ER positive before chemotherapy, conversion was seen in 8 cases accounting for change in ER status of 57%. Similarly 5 changes were seen in PR receptor expression

accounting for 62% of change. All the changes found were loss of expression of receptor after NACT means receptor positive cases became receptor negative.

Conclusion: Breast cancer subtypes are associated with the response to NACT. The response rates for the HE and TN subtypes were significantly higher than for the luminal subtypes. So it is mandatory for a patient with breast cancer who is scheduled for NACT should be assessed for the subtype of breast cancer before NACT, by using IHC, for planning treatment. This study also revealed that change in receptor status did occur after neo-adjuvant chemotherapy.

Key words

Neo-adjuvant Chemotherapy, Her-2 Receptor Status, Carcinoma Breast.

Introduction

Breast cancer is one of the commonest causes of death in many developed countries and is becoming frequent in developing countries like India as well. Breast cancer causes 519,000 deaths a year world-wide and about 900,000 women are diagnosed every year with breast cancer [1].

Breast cancer is relatively infrequent in Indian women and also occurs a decade earlier than in western women – the mean age of occurrence is about 42yrs in India as compared to 53years in white women. These differences are due to environmental rather than genetic factors [2].

A prognostic factor consists of any measurement available at the time of surgery that correlates with disease-free or overall survival in the absence of systemic adjuvant therapy and, as a result, is able to correlate with the natural history of the disease.

A predictive factor is any measurement associated with response to a given therapy.

Some factors, such as hormone receptors and HER-2/neu over-expression, are both prognostic and predictive [3].

Axillary lymph nodes are the principal prognostic factor in breast cancer [4], and their status guides systemic adjuvant therapy [5]. However, adjuvant chemotherapy is not the only method of treatment available. Currently, systemic therapy prior to surgery (neoadjuvant chemotherapy, NAC) is well established as a treatment for breast cancer.

The reliability of this therapy has been confirmed by the low index of tumor progression during chemotherapy, and its toxicity is similar to that found with adjuvant treatment. NAC improves surgical conditions even in patients with locally advanced disease [6].

In order to assess progression of the disease, the patient's age, histological tumor type, tumor size, degree of differentiation, estrogen and progesterone receptors (ER and PR, respectively) and HER-2/neu have been used as predictive factors.

ER, PR and HER-2/neu have by this time been extensively studied using immunohistochemical techniques in a search for predictive factors [7, 8].

Furthermore, identifying the predictive factors of response to primary systemic treatment may represent a potentially feasible clinical tool, since the future of neo-adjuvant treatment requires it to be individualized through target therapies [9, 10].

For instance, patients with tumors that overexpress HER-2/neu may benefit if trastuzumab is added to paclitaxel chemotherapy. Likewise, patients with tumors with positive ER may benefit from anti-estrogen therapy.

Biopsied tissue fragments are often the only specimens used to assess prognostic and

predictive markers prior to neoadjuvant systemic treatment because of the high agreement rates, which range from 0.77 to ~1 [11]. Since there is great variation in the histological and immunohistochemical findings in breast cancer, the possible changes between biopsies performed prior to and after NAC represent a challenge to the surgeon when a clinical decision is needed, as the effect of NAC on tumor marker status remains controversial [12].

Materials and methods

Cases of carcinoma breast requiring preoperative chemotherapy from January 2015 to January 2017 were recruited in to the study after informed consent.

Inclusion criteria: All patients with biopsy proven carcinoma breast who were eligible for neo adjuvant chemotherapy.

Exclusion criteria: Patients with Age above 70 years, age below 18 years and those not giving informed consent were excluded from the study.

Those patients who were subjected to neo adjuvant chemotherapy (with 4cycles of doxorubicin and cyclophosphamide and 4 cycles of paclitaxel) were assessed for hormonal receptor status in core needle biopsy sample by immunohistochemistry. ER (1:100, 1D5; Dako, Glostrup, Denmark), PR (1:200, PgR636; Dako), HER2 (DAKO Herceptest).

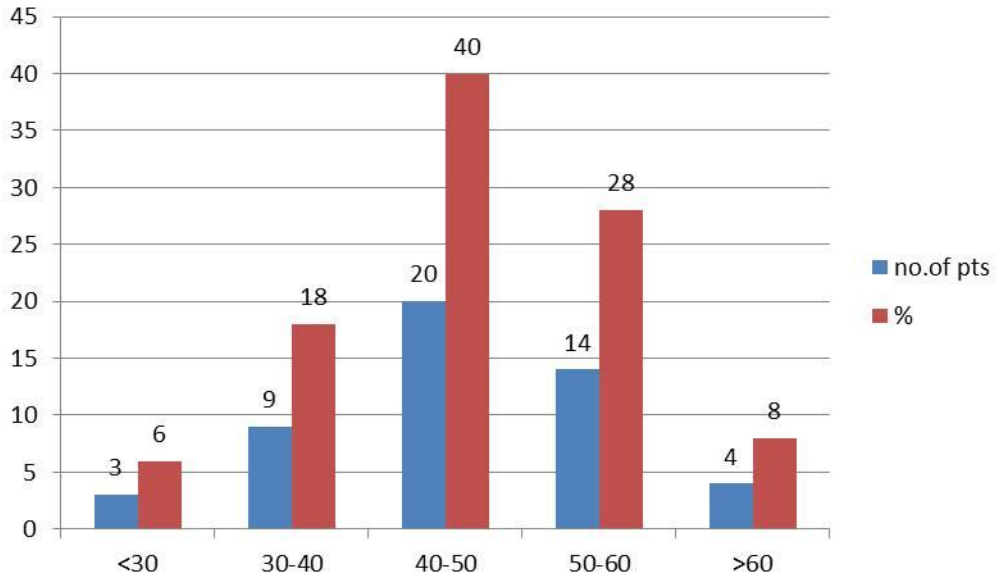
After completion of neo adjuvant chemotherapy and surgery hormonal receptor status was again evaluated in resected specimen with the same immunohistochemistry.

The data was analysed for any change in the receptor status.

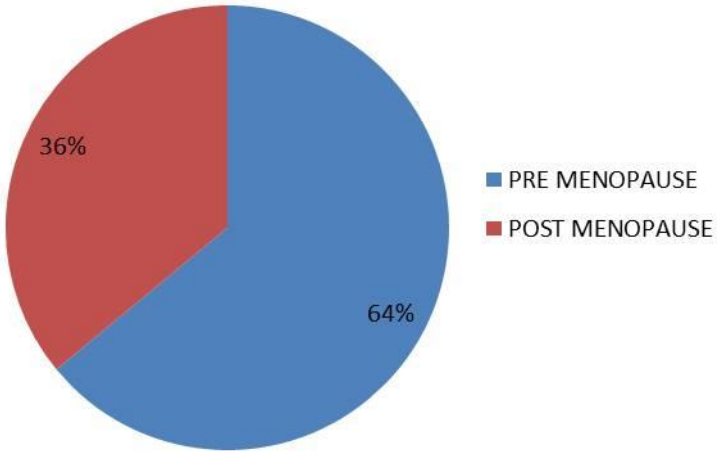
Results

In this study, the total of 50 cases of locally advanced breast cancers was included. Among them most of the cases belonged to the age group of 40 to 60. The extremes of ages (<30 and >60) comprised of only 14% of the cases (**Graph – 1**). Patient with locally advanced carcinoma breast in this study were categorised based on menopausal status. Post-menopausal age defined as women with cessation of menstrual cycle for a period of one year or age more than 50 years. In total of 50 cases, 32 patients were pre-menopausal which was accounting for 64% of cases, and remaining patients were post-menopausal (**Graph – 2**).

Graph - 1: Graphic representation of the demographic chart.



Graph – 2: Menopausal status.



Graph - 3: Pre chemotherapy receptor status.

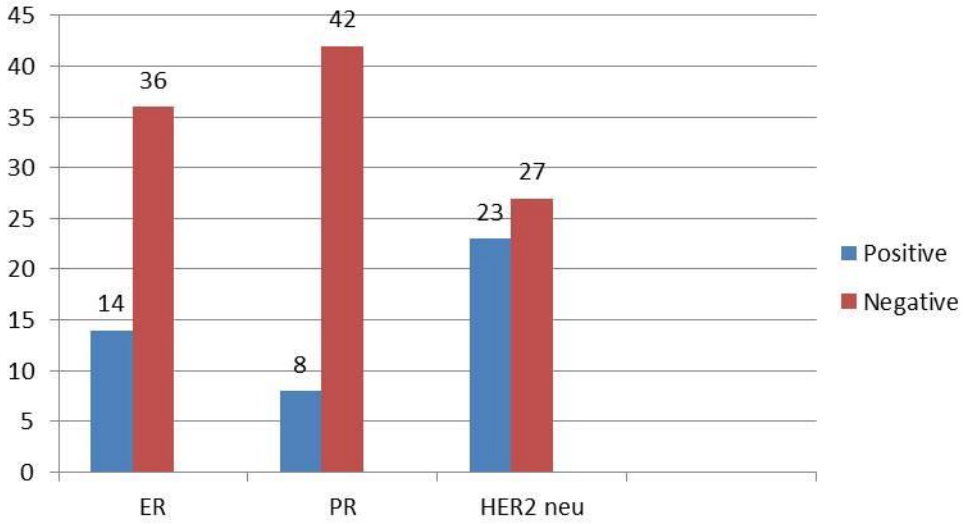


Table – 1: The receptor status in core biopsy.

Receptor status	Number	%
ER + PR+	8	16
ER+ PR-	6	12
ER- PR+	0	0
ER- PR-	36	72

Table - 2: The HER 2 NEU was categorised as positive if IHC score 3+ and negative for IHC score of 0, 1+ and 2+. FISH test was not done.

HER2 neu score	Number	%
0	21	42
+1	0	0
+2	6	12
+3	23	46

Table - 3: The response of CR, PR, SD, and PG.

Response	Number	%
CR	6	12
PR	42	84
SD	1	2
PG	1	2

Receptor status in core biopsy assessed by immunohistochemistry were categorised as ER PR positive or negative (**Graph – 3**). The receptor status in core biopsy was as per **Table – 1**. The HER 2 NEU was categorised as positive if IHC score 3+ and negative for IHC score of 0, 1+ and 2+. FISH test was not done (**Table – 2**).

Table - 4: Post chemo receptor status.

Receptor status	**Positive**	**Negative**
Estrogen	6	44
Progesterone	3	47
Her2neu	21	29
Receptor status	**Number**	**Percentage**
ER + PR+	3	6.8
ER+ PR-	3	6.8
ER- PR+	0	0
ER- PR-	38	86.4
HER2 neu status	**Number**	**Percentage**
0	24	54.5
1+	0	0
2+	4	9.1
3+	16	36.4

Table - 5: Change in receptor status after neo-adjuvant chemotherapy.

Changes in status	**Number**
Estrogen	8
Progestrerone	5
Her2neu	11

Response was evaluated both clinically and pathologically. Clinical response was evaluated as per Response Evaluation Criteria in Solid Tumors (RECIST v.1.0) developed by the World Health Organization, were categorised as Progression (PG), Stable (SD), Partial Response (PR), Complete Response (CR). Pathological complete response was noted in 6 cases which accounted for about 12 percent of all the cases. All the completely responded cases belonged to the triple negative group. Out of 20 cases of triple negative in this study complete response was seen in 6 cases accounting for about 30% (**Table – 3**). Post chemo receptor status was as per **Table – 4**.

In our study there were a total of 24 changes in the receptor status post chemotherapy altogether. Out of which 11 changes were seen in Her2neu group. Changes in the ER and PR group were accounted for 8 and 5 cases respectively (**Table – 5**).

All the changes in ER and PR receptor were seen as positive status being converted to negative status (**Table – 6**).

Table - 6: Change in ER, PR receptor status.

Receptor status	**+ → -**	**+ → +**	**- → +**	**- → -**
ER	8	6	0	36
PR	5	3	0	42

Table - 7: Change in ER, PR receptor status in post and pre chemo.

	Pre chemo	**Post chemo**	**Change**	**Change %**
ER positive	14	6	8	57.2%
PR positive	8	3	5	62.5%

Table – 8: Change in HER 2 NEU status after neo-adjuvant chemotherapy.

Receptor status	**+3 → 0**	**+2 → 0**	**0→ +2**	**0→ +3**
No. of cases	8	2	1	0

Out of 14 cases of ER positive before chemotherapy, conversion seen in 8 cases accounting for change in ER status of 57.2%. Similarly change in PR status accounted for about 62.5% (**Table – 7**). Change in HER 2 NEU status after neo-adjuvant chemotherapy was as per **Table – 8**.

Discussion

Breast cancer is a clinically heterogeneous disease. Histologically similar tumors may have different prognoses and may respond to therapy differently. It is believed that these differences in clinical behaviour are due to molecular differences between histologically similar tumors. The different molecular classes of breast cancer not only have different prognoses but also show distinct sensitivities to preoperative

chemotherapy. The basal-like and erbB2+ subtypes of breast cancer are more sensitive to paclitaxel- and doxorubicin containing preoperative chemotherapy than the luminal and normal-like cancers. Evidence from accumulated neo-adjuvant studies revealed that pCR provides a surrogate marker that is predictive for long-term clinical response and survival.

This study revealed that breast cancer subtypes are associated with the response to NACT. The pCR rates for the HE and TN subtypes were significantly higher than for the luminal subtypes.

Associations between the response to NACT and Receptor status

The overall pCR rate was 12%, similar to that in another study of Korean breast cancer patients [13]. In this study pCR rates were higher with neo-adjuvant treatment in patients with TNBC compared with those with non-TNBC (30% versus 0%). More recently, Von Minckwitz, et al. [14] presented the largest meta-analysis study to date comprising data from 6377 patients with operable or locally advanced, non-metastatic breast cancer having received neo-adjuvant anthracyclines, taxanes pCR rates were higher with neo-adjuvant treatment in patients with TNBC compared with those with non-TNBC (32% versus 10%).

Changes in the expression of IHC markers in the resection specimens after NACT

It is known that Significant changes in tumour morphology, grade occur following NACT but The impact of neo-adjuvant chemotherapy (NACT) on immunohistochemical markers in breast cancer specimens remains controversial.

We designed the current study to investigate the potential changes in estrogen receptor (ER), progesterone receptor (PR), HER2 expression before and after NACT. This study revealed that Changes in immunohistochemical markers before and after NACT do occur.

We found about 24 changes in estrogen receptor (ER), progesterone receptor (PR), HER2 expression among total of 50 cases accounting for 48% of change.

Changes in ER and PR Receptor Status

In breast cancer patients treated with neo-adjuvant chemotherapy it is proven that ER PR expression may significantly decreases after NACT possibly due to tumour heterogeneity. In addition, it is observed that the expression of HRs was associated with a worse response to NACT. Moreover, the present lowered cut-off points for ER and PR expression to 1%, in-creased the sensitivity, whereas most previous studies set the cut-off point at 10% which is responsible for wide variations of results among various studies. The topic of change in receptor status remains controversial.

In the present study, we considered cut-off points to 1% of tumour cells in biopsy specimens were positive for ER, PR, but in resection specimens, the tumour cells were entirely negative in most cases with expression loss after NACT.

Out of 14 cases of ER positive before chemotherapy, conversion seen in 8 cases accounting for change in ER status of 57%. Similarly 5 changes were seen in PR receptor expression accounting for 62% of change.

All the changes found were loss of expression of receptor after NACT means receptor positive cases became receptor negative.

As reviewed by Van de Ven, et al. [15] many previous studies revealed that the expression of HRs changes after NACT. About half of the studies that tested the ER and PR receptor status separately reported discordances of 2.5-37% and 5.9-51.7% respectively. Some studies concluded that the expression of HR did not change, but these studies had fewer patients than those that reached the opposite conclusion. Van de Ven, et al. [15] proposed several possible mechanisms for HR changes after NACT. One of the hypotheses is that a decrease in hormones such

as estrogen due to NACT might cause down regulation of HRs in the tumour and subsequently lead to estrogen-independent growth because ovarian dysfunction has been the most significant problem in premenopausal breast cancer patients treated with chemotherapy. However, loss of HR may be one of the mechanisms for the suppression of tumor progression by NACT rather than resistance to NACT.

Changes in HER2neu status

Although the concordance between HER2 overexpression detected by immunohistochemistry and HER2 gene amplification by FISH has been shown to be statistically significant , there are issues regarding consistency in immunohistochemistry testing that may affect results, including variable fixation, antigen retrieval methods, and observer analysis . In addition, FISH has been shown to be more reproducible than immunohistochemistry. It is unclear whether this change reflects response to therapy or a mechanism of resistance. It is possible that a change in HER2 status could reflect the heterogeneity of HER2 expression within the tumor suggesting that chemotherapy eliminateHER2-overexpressing clones leaving only HER2-negative tumor cells upon completion of therapy.

In this study, we assessed HER2 status only with immunohistochemistry both in the pre chemo and post chemotherapy and compared the IHC scores.

Regarding the changes in HER2neu status in this study which was accounting for majority of the changes were in about 11 cases. Majority of the change are HER2-positive tumors changed to HER2-negative about 10 cases accounting for 34% of change of HER2. Of the 72 HER2-positive tumors, 40% changed to HER2-negative, whereas only 3% of HER2-negative tumors changed to positive.

Harris, et al. reported a HER2 conversion rate of 32% in 18 patients with enough residual tissue to repeat HER2 testing by immunohistochemistry [16].

Conclusion

Hence we concluded that, ER, PR expression and the HER 2neu status in the resection specimen after NACT should be interpreted carefully because NACT tends to cause a decrease in the expression of these molecules and Until more comparable studies are being published, retesting the receptor status of the residual tumour after NACT should be considered in order to improve future tailored adjuvant therapies, and further research should be done in order to understand the clinical significance of this change in receptor status.

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