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# Effect of neoadjuvant chemotherapy on the expression of hormone receptors and Ki-67 in patients with locally advanced breast cancer

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## ABSTRACT

**Background:** Neoadjuvant chemotherapy (NACT) affects the tumor protein marker's expression and status. Changes in the expression of biomarkers during NACT may influence the clinical decision of adjuvant molecular and hormonal therapy. Hence, re-evaluation of receptor status has to be done after NACT to give a specific adjuvant therapy for patient benefit. **Aims and Objectives:** The aim of this study was to study the status of hormone receptors (HRs) – estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (Her-2/Neu), and Ki-67 in patients with locally advanced breast cancer (LABC) in both core needle biopsies and post-mastectomy specimens after administering neoadjuvant chemotherapy. **Materials and Methods:** The present study was conducted in the Department of Pathology in collaboration with the Department of Surgery, Pt B D Sharma PGIMS, Rohtak, for a period of 1 year. The study comprised 52 patients with LABC who received neoadjuvant chemotherapy and 20 control cases who did not receive neoadjuvant chemotherapy. Each case comprised two specimens: Trucut biopsy and a mastectomy specimen. **Results:** ER changed from positive to negative in 15.4% of the cases and from negative to positive in 1.9% of the cases. The overall change in ER was statistically significant (P = 0.020). For PR, 9.6% of the cases changed from positive to negative while none of the cases showed change from negative to positive. The overall change in PR was statistically significant (P = 0.025). For HER2/Neu, 19.2% of the cases changed from positive to negative, and 11.5% of the cases changed from negative to positive. The overall change in HER2/Neu was not statistically significant (P = 0.317). For Ki-67, 30% of patients changed from positive to negative while 11.5% of patients changed from negative to positive. The overall change in Ki-67 was statistically significant (P = 0.024). **Conclusion:** There was discordance in the HR status and proliferation marker after NACT in patients with breast cancer. The administration of NACT might be the main reason for the change in receptor status thus understanding the chemotherapy-induced biological conversion in the tumor cell behavior is strategically important in planning adjuvant endocrine therapy and for disease follow-up.

**Key words:** Breast cancer; Locally advanced; Neoadjuvant chemotherapy; Hormone receptors; Ki-67

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## INTRODUCTION

Locally advanced breast cancer (LABC) is a subset of BC characterized by the most advanced breast tumors in the absence of distant metastasis. The need to identify LABC as a separate group of BC arose in view of the high associated rate of locoregional and systemic failure (in the absence of distant metastasis at presentation) despite the best efforts of surgeons to remove locoregional spread of the tumor in its entirety. It was recognized that multimodality treatment

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(surgery, chemotherapy, and radiotherapy in combination with hormonal and targeted therapy if required) can significantly improve outcomes in this select group of patients.[1-3]

Neoadjuvant chemotherapy (NACT) is a standard of care for patients with inoperable or high-risk operable BC with the aim to reduce the extent of surgery. In addition, information on the response obtained at surgery is used not only to assess the long-term prognosis of patients but also as a short-term endpoint to evaluate the efficacy of established treatments in an individual patient or of innovative regimens within a clinical trial situation. Pathologic complete response is considered a surrogate efficacy endpoint generally correlated with favorable long-term outcome.[4,5]

Some studies revealed that NACT affects the tumor protein marker's expression and status. The classification of breast cancer (BC) subtypes based on tumor protein markers plays a very important role in systemic therapy and prognosis.[6]

Therefore, due to these changes; chemotherapy, endocrine, and/or targeted therapy cannot be made based only on the immunohistochemical (IHC) results obtained before NACT. Instead, systemic therapy should be guided by the results of multiple IHC from before and after NACT, and from eventual recurrences hence conversion of the hormone receptor (HR) status and human epidermal growth factor receptor 2 (HER2/Neu) status after NACT can be used to predict the prognosis of BC patients.[7]

This study was planned to determine the changes in estrogen receptor (ER), progesterone receptor (PR), HER2/Neu, and Ki-67 expression in core biopsy and post-mastectomy specimens in patients with LABC after NACT to assist in planning appropriate therapy for the patient based on HR status.

### Aim

To study the change in hormone receptor status in patients with locally advanced breast cancer recieving neoadjuvant chemotherapy.

### Objectives

1. To study the hormone receptor expression in core needle biopsies.
2. To study the hormone receptor expression in post mastectomy specimens after administering neo adjuvant chemotherapy to Breast cancer patients.
3. To compare the change in expression of these hormone receptors.

## MATERIALS AND METHODS

### Case selection

This longitudinal observational study was conducted in the Department of Pathology in collaboration with the Department of Surgery, Pt. B. D. Sharma PGIMS, Rohtak. The study was completed over a period of 1 year.

### Inclusion criteria

The study included 52 cases of patients with LABC who received neoadjuvant chemotherapy and 20 control cases who did not receive neoadjuvant chemotherapy.

### Exclusion criteria

Inadequate biopsies and patients who achieved pathological complete response after neoadjuvant chemotherapy with no residual tumor were excluded from the study.

### Morphological evaluation

Each case comprised two specimens: Trucut biopsy and mastectomy specimen after neoadjuvant therapy. Both tissues were processed for preparation of tissue sections from formalin-fixed, paraffin-embedded tissue block. The tissues were fixed in 10% formalin, processed for histopathological examination, and sections obtained were stained with hematoxylin and eosin (H&E) stain as per standard protocol. Grading of the tumor was done according to the modified Bloom-Richardson grading system. This grading scheme is based on three morphologic features including the degree of tumor tubule formation, tumor mitotic activity, and nuclear pleomorphism of tumor cells (nuclear grade). The size of tumor and lymph node status was seen and the prognostic index was calculated according to Nottingham prognostic index. Staging of the tumor was done according to tumor, node, metastasis (TNM) staging system.

### IHC analysis

Representative sections from each case (trucut biopsy and mastectomy specimen after neoadjuvant therapy) were subjected to IHC staining for ER, PR, HER-2/Neu, and Ki-67. IHC expression was assessed and correlated with other clinicopathological parameters. Normal breast tissue served as a positive control for all IHC markers and negative control was obtained by substituting the primary antibody with the antibody of non-specific relevance.

ER and PR are nuclear receptors. IHC scoring for ER and PR was done by Allred system of scoring. In Allred system of scoring, Score 0–5 is given to the cells depending on the proportion of cells which are stained (proportion score [PS]), and Score 0–3 is given depending on the intensity of staining (intensity score [IS]). By adding the PS and IS, the final Allred score (PS+IS=AS) is calculated for ER and

PR. The two scores are added together for a final score (0–8). Patients with scoring three or more were regarded as ER/PR positive.

HER-2/Neu is a cell membrane receptor and depending on the intensity of staining a score of 0–3 is given to the cells.

0 Negative: No staining is observed, or membrane staining is observed in <10% of the tumor cells.

1+ Negative: A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

2+ Weakly positive or equivocal: A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

3+ Strongly positive: A strong complete membrane staining is observed in more than 30% of the tumor cells.

Equivocal (2+) cases to be confirmed by fluorescence *in situ* hybridization.

Ki-67 protein in humans is encoded by the MK167 gene and is a cellular marker for proliferation. This is a nuclear protein and is expressed in proliferating cells but is not detected in resting cells. The Ki-67 expression as detected by IHC is one of the most reliable prognostic and predictive markers. The most commonly used method to detect Ki-67 positivity is by staining with MIB-1 antibody. The proliferation rate is shown as the number of Ki67-positive cells with nuclear staining in 100 carcinoma cells per section. In accordance with the study by Fasching et al., a proportion of ≥13% positively stained cells was used as the cutoff point for Ki67 status in our study.[8]

### Statistical analysis

The collected data were analyzed with the help of a software package (Statistical Package for the Social Sciences version 24.0).

All the data enlisted in the investigation pro forma (name, age, sex, CR no, clinical diagnosis, and history) were collected. Both cases and controls were assessed for different clinicopathological parameters, cases were compared pre- and post-NACT for HR expression. The association was tested using Chi-square, and correlation was assessed using Spearman test. P<0.05 was accepted as statistically significant.

## RESULTS

The study comprised 52 cases of patients with LABC who received neoadjuvant chemotherapy and 20 control cases who did not receive neoadjuvant chemotherapy. Each case comprised two specimens: Trucut biopsy and a mastectomy specimen.

**Table 1: Distribution of cases and controls according to age**

| Age (years) | Cases (n=52) (%) | Controls (n=20) (%) |
|---|---|---|
| <35 years | 3 (5.7) | 5 (25) |
| 35–45 years | 16 (30.8) | 4 (20) |
| 45–55 years | 19 (36.5) | 6 (30) |
| 55–65 years | 8 (15.4) | 5 (25) |
| >65 years | 6 (11.5) | 0 (0) |
| Total | 52 (100) | 20 (100) |

### Clinical parameters

The mean age among cases was 50.85±11.04 years. The mean age among controls was 45.50±11.87 years. A maximum number of cases (36%) fall in 45–55 years age group. A maximum number of controls (30%) fall in 45–55 years age group (Table 1).

Maximum number of cases (40.4%) received three and four cycles of NACT each. About 15.4% of the cases received six cycles of NACT. About 1.9% of the cases received eight and nine cycles of NACT each. Maximum number of cases (59.6%) involved the left breast. Maximum number of controls (65.0%) involved the right breast. In total 71.2% of the cases and 70% of the controls involved the upper quadrant, out of which the maximum number of participants (38.5% of the cases and 60% of the controls) involved the upper outer quadrant.

### Morphological evaluation

All the cases and controls were divided according to tumor size into three subgroups (<2 cm, 2–5 cm and >5 cm). Maximum number of cases (73.1%) and maximum number of controls (55%) had tumor size 2–5 cm. The mean tumor size among the cases was 4.69±3.39 cm. The mean tumor size among the control group was 3.85±1.70 cm. The tumor size among the case group ranged from 1 to 17 cm. The tumor size among the control group ranged from 1 to 7 cm.

All the cases and controls were graded using Nottingham modification of Bloom-Richardson grading system. Majority (59.6%) of the cases were of Grade 2 (Moderately differentiated). Majority (55.0%) of the controls were of Grade 1 (Well differentiated). Majority of the cases (69.2%) were of stage T2. Majority of the controls (90.0%) were of stage T2. Majority (40.4%) of the cases and the majority (50.0%) of the controls were of stage N0 (did not show any lymph node metastasis). Cases were divided into three prognostic categories according to NPI Score:

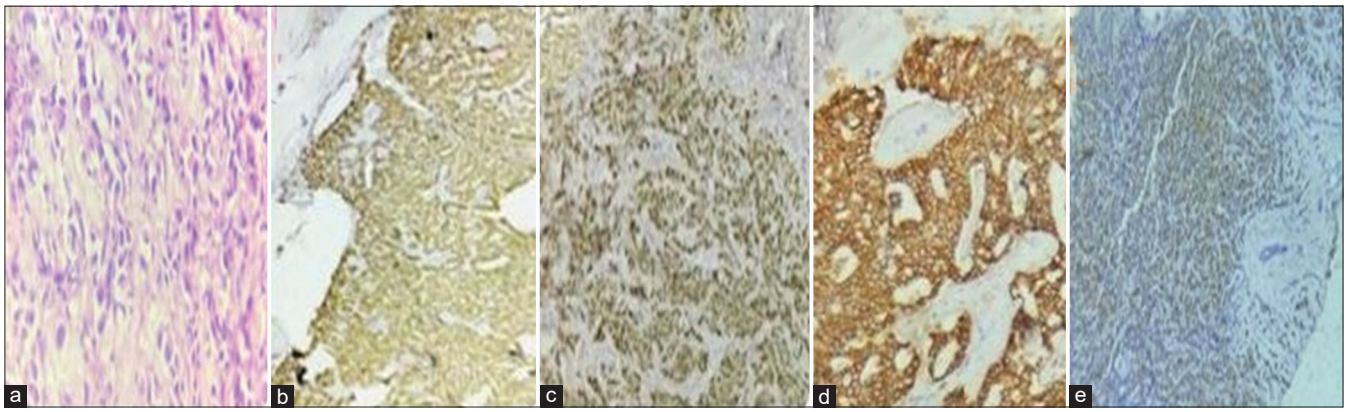


**Figure 1:** Trucut biopsy pre NACT: (a) infiltrating ductal carcinoma grade 2 (H&E stain, ×400), (b) ER positive (IHC stain, ×100), (c) PR positive (IHC stain, ×100), (d) HER2/Neu positive (IHC stain, ×200), (e) High Ki67 (IHC stain, ×100**).** ER**:** Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor, NACT: Neoadjuvant chemotherapy, NPI: Nottingham prognostic index. IHC: Immunohistochemical

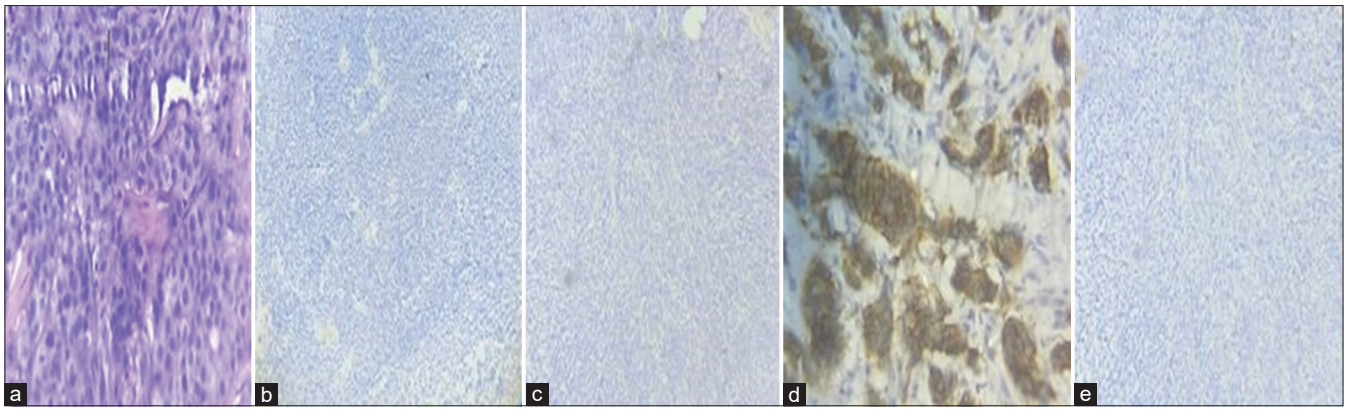


**Figure 2:** Modified radical mastectomy specimen post-NACT: (a) infiltrating ductal carcinoma Grade 3 (H&E stain, ×400), (b) ER negative (IHC stain, ×100), (c) PR negative (IHC stain, ×100), (d) HER2/Neu positive (IHC stain, ×200), (e) Low Ki67 (IHC stain, ×100). ER**:** Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor, NACT: Neoadjuvant chemotherapy, NPI: Nottingham prognostic index. IHC: Immunohistochemical

Good (<3.4), moderate (3.4–5.4), and poor prognosis (>5.4). Majority (57.7%) of the cases group had moderate prognosis. Majority (60%) of the controls had moderate prognosis.

## IHC analysis (Figures 1 and 2)

### *Comparison of ER, PR, HER-2/Neu, and Ki-67 expression among cases pre- and post-NACT*

For ER, 8 (15.4%) patients changed from positive to negative. One (1.9%) patient changed from negative to positive. The overall change in ER was statistically significant (McNemar's test: $\chi^2$=5.444, P=0.020). For PR, 5 (9.6%) patients changed from positive to negative. No patient showed a change from negative to positive. The overall change in PR was statistically significant (McNemar's test: $\chi^2$=5.000, P=0.025). For HER-2/Neu, 10 (19.2%) patients changed from positive to negative. Six (11.5%) patients changed from negative to positive. The overall change in HER-2/Neu was not statistically significant (McNemar's test: $\chi^2$=1.000, P=0.317). For Ki-67, 16 (30%) patients changed from positive to negative, and 6 (11.5%) patients changed from negative to positive. The overall change in ki67 was statistically significant (McNemar's test: $\chi^2$=1.000, P=0.024) (Table 2).

**Table 2: Change in expression of ER, PR, HER2/Neu, and Ki-67 among cases pre and post–NACT**

| Hormone receptor | Pre-NACT (in percentage) | Post-NACT (in percentage) | P-value |
|---|---|---|---|
| ER | | | |
| Positive | 48.1 | 34.6 | 0.020 |
| Negative | 51.9 | 65.4 | |
| PR | | | |
| Positive | 48.1 | 38.5 | 0.025 |
| Negative | 51.9 | 61.5 | |
| HER2/Neu | | | |
| Positive | 36.5 | 28.8 | 0.317 |
| Negative | 63.5 | 71.2 | |
| Ki67 | | | |
| Positive | 69.3 | 50 | 0.024 |
| Negative | 30.7 | 50 | |

ER: Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor, NACT: Neoadjuvant chemotherapy

### *Comparison of ER, PR, HER-2/Neu, and Ki-67 expression among control pre- and post-NACT*

For ER, 2 (10.0%) patients changed from positive to negative, and 1 (5.0%) patient changed from negative to positive. The overall change in ER was not statistically significant (McNemar's test: $\chi^2$=0.333, P=0.564). For PR, 2 (10.0%) patients changed from negative to positive and none patients changed from positive to negative. The overall change in PR was not statistically significant (McNemar's test: $\chi^2$=2.000, P=0.157). For HER-2/Neu, 1 (5.0%) patient changed from positive to negative, and 2 (10.0%) patients changed from negative to positive. The overall change in HER/Neu was not statistically significant (McNemar's test: $\chi^2$=0.333, P=0.564). For Ki-67, 2 (10.0%) patients changed from positive to negative, and 2 (10.0%) patients changed from negative to positive. The overall change in ki67 was statistically significant (McNemar's test: $\chi^2$=0.333, P=0.564).

**Table 3: Association between changes in ER (post-NACT) and other clinicopathological parameters among the cases**

| Parameters | Change in ER (post-treatment) | | | | P-value |
|---|---|---|---|---|---|
| | Became negative (n=8) | Became positive (n=1) | Remained negative (n=26) | Remained positive (n=17) | |
| Age (years) | 50.25±10.82 | 88.00±0 | 48.88±10.31 | 51.94±8.77 | 0.271 |
| Side of breast (%) | | | | | 0.558 |
| Right | 4 (50.0) | 0 (0.0) | 12 (46.2) | 5 (29.4) | |
| Left | 4 (50.0) | 1 (100.0) | 14 (53.8) | 12 (70.6) | |
| Quadrant of breast(%) | | | | | 0.486 |
| Central | 3 (37.5) | 0 (0.0) | 6 (23.1) | 2 (11.8) | |
| Lower inner | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (11.8) | |
| Lower outer | 0 (0.0) | 0 (0.0) | 1 (3.8) | 1 (5.9) | |
| Upper inner | 1 (12.5) | 1 (100.0) | 8 (30.8) | 7 (41.2) | |
| Upper outer | 4 (50.0) | 0 (0.0) | 11 (42.3) | 5 (29.4) | |
| Tumor size (cm) | 5.62±4.87 | 3.10±0 | 4.54±3.23 | 4.59±3.04 | 0.964 |
| Lymph nodes harvested | 12.12±8.24 | 16.00±0 | 10.15±3.76 | 10.41±5.66 | 0.638 |
| Lymph nodes positive | 7.88±8.58 | 1.00±0 | 2.15±3.54 | 3.18±4.19 | 0.196 |
| Lymph node positivity (%) | 55.61±39.62 | 6.25±0 | 21.18±33.06 | 30.94±36.17 | 0.130 |
| Tumor grade (%) | | | | | 0.916 |
| Grade 1 | 2 (25.0) | 0 (0.0) | 5 (19.2) | 6 (35.3) | |
| Grade 2 | 5 (62.5) | 1 (100.0) | 16 (61.5) | 9 (52.9) | |
| Grade 3 | 1 (12.5) | 0 (0.0) | 5 (19.2) | 2 (11.8) | |
| T Stage (%) | | | | | 0.707 |
| T1 | 1 (12.5) | 0 (0.0) | 3 (11.5) | 0 (0.0) | |
| T2 | 4 (50.0) | 1 (100.0) | 17 (65.4) | 14 (82.4) | |
| T3 | 2 (25.0) | 0 (0.0) | 4 (15.4) | 2 (11.8) | |
| T4 | 1 (12.5%) | 0 (0.0%) | 2 (7.7%) | 1 (5.9%) | |
| N Stage (%) | | | | | 0.238 |
| N0 | 2 (25.0) | 0 (0.0) | 14 (53.8) | 5 (29.4) | |
| N1 | 1 (12.5) | 1 (100.0) | 6 (23.1) | 7 (41.2) | |
| N2 | 1 (12.5) | 0 (0.0) | 3 (11.5) | 2 (11.8) | |
| N3 | 4 (50.0) | 0 (0.0) | 3 (11.5) | 3 (17.6) | |
| NPI score | 4.55±0.78 | 4.60±0 | 4.71±1.38 | 4.64±1.22 | 0.932 |
| NPI score category (%) | | | | | 0.865 |
| <3.4 | 1 (12.5) | 0 (0.0) | 3 (11.5) | 3 (17.6) | |
| 3.4–5.4 | 6 (75.0) | 1 (100.0) | 15 (57.7) | 8 (47.1) | |
| >5.4 | 1 (12.5) | 0 (0.0) | 8 (30.8) | 6 (35.3) | |
| Change in PR (post-treatment)*** (%) | | | | | <0.00 |
| Became negative | 5 (62.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Became positive | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Remained negative | 0 (0.0) | 0 (0.0) | 26 (100.0) | 1 (5.9) | |
| Remained positive | 3 (37.5) | 1 (100.0) | 0 (0.0) | 16 (94.1) | |
| Change in HER-2/Neu (post-treatment) (%) | | | | | 0.58 |
| Became negative | 1 (12.5) | 0 (0.0) | 7 (26.9) | 2 (11.8) | |
| Became positive | 1 (12.5) | 0 (0.0) | 2 (7.7) | 3 (17.6) | |
| Remained negative | 6 (75.0) | 1 (100.0) | 13 (50.0) | 7 (41.2) | |
| Remained positive | 0 (0.0) | 0 (0.0) | 4 (15.4) | 5 (29.4) | |
| Change in KI67 (%) (post-treatment) | −23.75±32.38 | 0.00±0 | −11.54±20.40 | −4.47±9.82 | 0.241 |

***Significant at P<0.05, Change in PR (post-NACT) was significantly associated (P<0.05) with Change in ER (Post-NACT). ER: Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor, NACT: Neoadjuvant chemotherapy, NPI: Nottingham prognostic index

**Table 4: Association between changes in PR (post-NACT) and other clinicopathological parameters among the cases**

| Parameters | Change in PR (post-treatment) | | | | P-value |
|---|---|---|---|---|---|
| | Became negative (n=5) | Became positive (n=0) | Remained negative (n=27) | Remained positive (n=20) | |
| Age (years) | 48.20±9.93 | - | 49.63±10.82 | 53.15±11.67 | 0.553 |
| Side of breast (%) | | | | | 0.341 |
| Right | 3 (60.0) | 0 (0.0) | 12 (44.4) | 6 (30.0) | |
| Left | 2 (40.0) | 0 (0.0) | 15 (55.6) | 14 (70.0) | |
| Quadrant of breast(%) | | | | | 0.683 |
| Central | 2 (40.0) | 0 (0.0) | 6 (22.2) | 3 (15.0) | |
| Lower inner | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (10.0) | |
| Lower outer | 0 (0.0) | 0 (0.0) | 1 (3.7) | 1 (5.0) | |
| Upper inner | 1 (20.0) | 0 (0.0) | 8 (29.6 | 8 (40.0 | |
| Upper outer | 2 (40.0) | 0 (0.0) | 12 (44.4) | 6 (30.0) | |
| Tumor size (cm) | 3.20±1.10 | - | 4.52±3.17 | 5.30±3.97 | 0.300 |
| Lymph nodes harvested | 14.80±8.38 | - | 10.19±3.69 | 10.25±5.91 | 0.561 |
| Lymph nodes positive | 10.20±9.60 | - | 2.11±3.48 | 3.30±4.33 | 0.064 |
| Lymph node positivity (%) | 54.31±37.42 | - | 20.73±32.50 | 34.82±38.58 | 0.094 |
| Tumor grade (%) | | | | | 0.913 |
| Grade 1 | 1 (20.0) | 0 (0.0) | 6 (22.2) | 6 (30.0) | |
| Grade 2 | 4 (80.0) | 0 (0.0) | 16 (59.3) | 11 (55.0) | |
| Grade 3 | 0 (0.0) | 0 (00) | 5 (18.5) | 3 (15.0) | |
| T stage (%) | | | | | 0.609 |
| T1 | 1 (20.0) | 0 (0.0) | 3 (11.1) | 0 (0.0) | |
| T2 | 3 (60.0) | 0 (0.0) | 18 (66.7) | 15 (75.0) | |
| T3 | 1 (20.0) | 0 (0.0) | 4 (14.8) | 3 (15.0) | |
| T4 | 0 (0.0) | 0 (0.0) | 2 (7.4) | 2 (10.0) | |
| N stage (%) | | | | | 0.296 |
| N0 | 1 (20.0) | 0 (0.0) | 14 (51.9) | 6 (30.0) | |
| N1 | 1 (20.0) | 0 (0.0) | 7 (25.9) | 7 (35.0) | |
| N2 | 0 (0.0) | 0 (0.0) | 3 (11.1) | 3 (15.0) | |
| N3 | 3 (60.0) | 0 (0.0) | 3 (11.1) | 4 (20.0) | |
| NPI score | 4.54±0.99 | - | 4.60±1.46 | 4.77±0.91 | 0.923 |
| NPI score category (%) | | | | | 1.000 |
| <3.4 | 1 (20.0) | 0 (0.0) | 4 (14.8) | 2 (10.0) | |
| 3.4–5.4 | 3 (60.0) | 0 (0.0) | 15 (55.6) | 12 (60.0) | |
| >5.4 | 1 (20.0) | 0 (0.0) | 8 (29.6) | 6 (30.0) | |
| Change in ER (post-treatment)*** (%) | | | | | <0.00 |
| Became negative | 5 (100.0) | 0 (0.0) | 0 (0.0) | 3 (15.0) | |
| Became positive | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (5.0) | |
| Remained negative | 0 (0.0) | 0 (0.0) | 26 (96.3) | 0 (0.0) | |
| Remained positive | 0 (0.0) | 0 (0.0) | 1 (3.7) | 16 (80.0) | |
| Change in HER2/Neu (post-treatment) (%) | | | | | 0.694 |
| Became negative | 1 (20.0) | 0 (0.0) | 7 (25.9) | 2 (10.0) | |
| Became positive | 0 (0.0) | 0 (0.0) | 3 (11.1) | 3 (15.0) | |
| Remained negative | 4 (80.0) | 0 (0.0) | 13 (48.1) | 10 (50.0) | |
| Remained positive | 0 (0.0) | 0 (0.0) | 4 (14.8) | 5 (25.0) | |
| Change in KI67 (%) (post-treatment) | −23.00±29.92 | - | −11.22±20.07 | −7.40±18.36 | 0.137 |

***Significant at P<0.05, Change in ER (post-NACT) was significantly associated (P<0.05) with Change in PR (Post-NACT). ER: Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor, NACT: Neoadjuvant chemotherapy, NPI: Nottingham prognostic index

Changes in expression ER, PR, HER-2/Neu, and Ki-67 (post-NACT) in association with other clinicopathological parameters among the cases were also tabulated (Tables 3-6).

## DISCUSSION

The fact that chemotherapeutic agents cause changes on components of the tumor cells is known since the 1960s. First, Waller demonstrated changes such as enlargement of the nucleus, swelling of the cytoplasm, and vacuolization in the cytoplasm/nucleus in tumor cells following systemic administration of busulphan. Since changes in the molecular properties of cancer cells may affect the tumor behavior and therefore the treatment plan to be followed, studies investigating how the chemotherapeutic agents affect tumor grade, receptor properties of tumor cells, and tumor proliferation rate have been increasing in number recently.[9,10]

**Table 5: Association between changes in HER2/Neu (post-NACT) and other clinicopathological parameters among the cases**

| Parameters | Change in HER2/Neu (post-treatment) | | | | P-value |
|---|---|---|---|---|---|
| | Became negative (n=10) | Became positive (n=6) | Remained negative (n=27) | Remained positive (n=9) | |
| Age (years) | 51.40±7.44 | 49.17±10.61 | 52.00±13.10 | 47.89±8.30 | 0.648 |
| Side of breast (%) | | | | | 0.228 |
| Right | 2 (20.0) | 1 (16.7) | 14 (51.9) | 4 (44.4) | |
| Left | 8 (80.0) | 5 (83.3) | 13 (48.1 | 5 (55.6) | |
| Quadrant of breast(%) | | | | | 0.560 |
| Central | 2 (20.0) | 0 (0.0) | 8 (29.6) | 1 (11.1) | |
| Lower inner | 1 (10.0) | 1 (16.7) | 0 (0.0) | 0 (0.0) | |
| Lower outer | 0 (0.0) | 0 (0.0) | 2 (7.4) | 0 (0.0) | |
| Upper inner | 3 (30.0) | 2 (33.3) | 7 (25.9) | 5 (55.6) | |
| Upper outer | 4 (40.0) | 3 (50.0) | 10 (37.0) | 3 (33.3) | |
| Tumor size (cm) | 5.00±2.27 | 3.75±1.78 | 4.69±3.72 | 5.00±4.42 | 0.562 |
| Lymph nodes harvested | 12.80±4.37 | 9.67±2.66 | 10.96±5.73 | 8.00±5.20 | 0.277 |
| Lymph nodes positive | 5.10±5.38 | 0.67±1.21 | 3.85±5.75 | 1.67±3.20 | 0.217 |
| Lymph node positivity (%) | 40.52±36.80 | 5.36±9.41 | 36.09±40.16 | 12.89±22.75 | 0.221 |
| Tumor grade (%) | | | | | 0.488 |
| Grade 1 | 2 (20.0) | 3 (50.0) | 6 (22.2) | 2 (22.2) | |
| Grade 2 | 5 (50.0) | 3 (50.0) | 16 (59.3) | 7 (77.8) | |
| Grade 3 | 3 (30.0) | 0 (0.0) | 5 (18.5) | 0 (0.0) | |
| T stage (%) | | | | | 0.221 |
| T1 | 0 (0.0) | 1 (16.7) | 1 (3.7) | 2 (22.2) | |
| T2 | 6 (60.0) | 4 (66.7) | 22 (81.5) | 4 (44.4) | |
| T3 | 3 (30.0) | 1 (16.7) | 2 (7.4) | 2 (22.2) | |
| T4 | 1 (10.0) | 0 (0.0) | 2 (7.4) | 1 (11.1) | |
| N stage (%) | | | | | 0.608 |
| N0 | 3 (30.0) | 4 (66.7) | 11 (40.7) | 3 (33.3) | |
| N1 | 2 (20.0) | 2 (33.3) | 7 (25.9) | 4 (44.4) | |
| N2 | 3 (30.0) | 0 (0.0) | 2 (7.4) | 1 (11.1) | |
| N3 | 2 (20.0) | 0 (0.0) | 7 (25.9) | 1 (11.1) | |
| NPI score*** | 5.12±1.04 | 3.47±1.02 | 4.71±1.27 | 4.78±1.02 | 0.047 |
| NPI score category (%) | | | | | 0.387 |
| <3.4 | 1 (10.0) | 2 (33.3) | 3 (11.1) | 1 (11.1) | |
| 3.4–5.4 | 4 (40.0) | 4 (66.7) | 17 (63.0) | 5 (55.6) | |
| >5.4 | 5 (50.0) | 0 (0.0) | 7 (25.9) | 3 (33.3) | |
| Change in ER (post-treatment) (%) | | | | | 0.588 |
| Became negative | 1 (10.0) | 1 (16.7) | 6 (22.2) | 0 (0.0) | |
| Became positive | 0 (0.0) | 0 (0.0) | 1 (3.7) | 0 (0.0) | |
| Remained negative | 7 (70.0) | 2 (33.3) | 13 (48.1) | 4 (44.4) | |
| Remained positive | 2 (20.0) | 3 (50.0) | 7 (25.9) | 5 (55.6) | |
| Change in PR (post-treatment) (%) | | | | | 0.694 |
| Became negative | 1 (10.0) | 0 (0.0) | 4 (14.8) | 0 (0.0) | |
| Became positive | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Remained negative | 7 (70.0) | 3 (50.0) | 13 (48.1) | 4 (44.4) | |
| Remained positive | 2 (20.0) | 3 (50.0) | 10 (37.0) | 5 (55.6) | |
| Change in KI67 (%) (post-treatment) | −7.80±18.51 | −2.00±22.18 | −13.22±23.01 | −13.22±13.07 | 0.514 |

***Significant at P<0.05, NPI Score was significantly associated (P<0.05) with Change in HER2/Neu (post-treatment). ER: Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor, NACT: Neoadjuvant chemotherapy, NPI: Nottingham prognostic index

In this study, we examined the qualitative and quantitative changes in ER, PR, HER-2, and Ki-67 in BC patients receiving NACT. Various clinicopathological parameters along with the changes in the receptor status in trucut biopsy and mastectomy specimens among both the cases and controls were analyzed and then compared with the findings in other similar studies (Table 7).

In our study, 48.1% of the cases were positive for ER and PR while 51.9% were negative for ER and PR in trucut biopsy specimens whereas in modified radical mastectomy (MRM) specimens 34.6% and 38.5% of the cases were positive and 65.4% and 61.5% were negative for ER and PR, respectively.

Our findings were in concordance with other studies like Peng et al.,[13] where positivity for ER decreased from 66.1% to 56.2% and PR decreased from 48.2% to 37.5%.

In our study, 36.5% of the cases were positive for HER-2/Neu and 63.5% were negative in trucut biopsy

**Table 6: Association between changes in Ki-67 (post-NACT) and other clinicopathological parameters among the cases**

| Parameters | Change in Ki-67 (%) (post-treatment) | P-value |
|---|---|---|
| Age (years) | Correlation coefficient (rho)=0.14 | 0.332 |
| Side of breast | | 0.553 |
| Right | −14.76±25.40 | |
| Left | −8.26±16.33 | |
| Quadrant of breast | | 0.893 |
| Central | −11.09±13.40 | |
| Lower inner | −13.50±14.85 | |
| Lower outer | −6.00±1.41 | |
| Upper inner | −7.88±16.51 | |
| Upper outer | −13.55±27.82 | |
| Tumor size (cm) | Correlation Coefficient (rho)=0.06 | 0.677 |
| Lymph nodes harvested | Correlation Coefficient (rho)=0 | 0.975 |
| Lymph nodes positive | Correlation Coefficient (rho)=0.02 | 0.885 |
| Lymph node positivity (%) | Correlation Coefficient (rho)=0.03 | 0.860 |
| Tumor grade | | 0.149 |
| Grade 1 | −1.85±11.22 | |
| Grade 2 | −13.13±18.86 | |
| Grade 3 | −16.88±33.27 | |
| T Stage | | 0.065 |
| T1 | −27.50±12.58 | |
| T2 | −9.11±21.46 | |
| T3 | −5.50±17.62 | |
| T4 | −21.00±16.45 | |
| N Stage | | 0.997 |
| N0 | −9.90±16.52 | |
| N1 | −11.27±22.88 | |
| N2 | −9.00±24.45 | |
| N3 | −13.50±24.84 | |
| NPI Score | Correlation Coefficient (rho)=−0.16 | 0.244 |
| NPI Score Category | | 0.325 |
| <3.4 | −2.14±2.12 | |
| 3.4–5.4 | −12.93±23.23 | |
| >5.4 | −10.87±19.08 | |
| Ki67 (%) (Pre-treatment)*** | Correlation coefficient (rho)=−0.73 | <0.001 |
| Change in ER (post-treatment) | | 0.241 |
| Became negative | −23.75±32.38 | |
| Became positive | 0.00±0 | |
| Remained negative | −11.54±20.40 | |
| Remained positive | −4.47±9.82 | |
| Change in PR (post-treatment) | | 0.137 |
| Became negative | −23.00±29.92 | |
| Became positive | – | |
| Remained negative | −11.22±20.07 | |
| Remained positive | −7.40±18.36 | |
| Change in HER2/Neu (post-treatment) | | 0.514 |
| Became negative | −7.80±18.51 | |
| Became positive | −2.00±22.18 | |
| Remained negative | −13.22±23.01 | |
| Remained positive | −13.22±13.07 | |

***Significant at P<0.05, Ki67 (%) (pre-treatment) was significantly associated (P<0.05) with change in KI67 (%) (post-treatment). ER: Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor, NACT: Neoadjuvant chemotherapy, NPI: Nottingham prognostic index

specimens whereas 28.8% of the cases were positive and 71.2% were negative in the MRM specimens. The expression of HER2/Neu was variable in various studies such as those conducted by Peng et al.,[13] Tacca et al.,[11] where positivity of HER2/Neu changed from 42% to 32.1% and 36.2% to 38.3%, respectively.

In our study, 69.3% of the cases were positive for Ki-67 and 30.7% of the cases were negative in trucut biopsy specimen, and in MRM specimen, 50% of the cases were positive, and 50% of the cases were negative which was in concordance to Peng et al., where ki67 positivity changed from 75.9% to 41.1%.

The discordance in HR status between core needle biopsies (CNB) and excision specimens has been reported in patients given neoadjuvant therapy but these results have not been consistent. Various studies[11-16]

**Table 7: Hormone receptor expression in cases among various studies**

| Studies | ER | PR | HER-2/Neu | KI67 |
|---|---|---|---|---|
| Tacca et al.[11] | Hormone receptor 42% from negative to positive 13% from positive to negative Significant | - | - | - |
| Hirata et al.[12] | Positive to negative – 8.2% Negative to positive 7.9% overall change 14.9% Significant | Overall change 29.1% Significant | Positive to negative – 6% Negative to positive – 3.5% Overall change 9.5% Significant | - |
| Peng et al.[13] | Positive to negative –16.1% Negative to positive – 6.2% Overall change – 22.3% Significant | Positive to negative –19.6% Negative to positive – 9% Overall change – 28.6% Significant | Positive to negative – 15.2% Negative to positive – 5.3% Overall change – 20.5% Significant | Positive to negative – 37.5% Negative to positive – 2.7% Overall change – 40.2% Significant |
| Wu et al.[14] | Negative to positive – 7.7% Overall change – 15.2% Not significant | Negative to positive – 26.9% Overall change – 26.9% Not significant | - | Overall change 44.8% Not significant |
| Present study | Positive to negative – 15.4% Negative to positive – 1.9% Significant | Positive to negative – 9.6% Negative to positive – none Significant | Positive to negative – 19.2% Negative to positive – 11.5% Not Significant | Positive to negative – 30% Negative to positive – 11.5% Significant |

-: Not available. ER: Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor

have shown a wide range of discordance ranging from 2% to 44.8%.

Wu et al.,[14] found a negative to positive ER switch in 7.7% of cases while Dede et al.,[15] concluded that a change in ER from positive to negative was seen in 5.7% of cases. According to Peng et al.,[13] positive-to-negative conversions was seen in 16.1% of the cases and negative to positive in 6.2% of the cases. The overall conversion was seen in 22.3% of the cases which was statistically significant. Similarly, Ramteke et al.,[16] found conversion from positive to negative in 15% of the cases.

In concordance to our study, Peng et al.,[13] and Wu et al.,[14] found that PR positivity decreased from 66.1% to 56.2% and 51% to 42.6%, respectively. Overall change by Wu et al.,[14] was found to be 26.9% while by Hirata et al.,[12] was found to be 29.1% which was statistically significant. However, in the study conducted by Dede et al.,[15] 21.1% of cases converted from positive to negative and 9% from negative to positive which was not significant.

In a study conducted by Peng et al.,[13] the positive rate of HER2 decreased from 42.0% to 32.1% (P=0.04) which was statistically significant. Dede et al.,[15] also reported a change in the expression of HER2/Neu with IHC methods in addition to HR changes in their study however their results were not statistically significant.

Majority of the studies reported a significant decrease in Ki-67 expression after NACT. In a study conducted by Peng et al.,[13] the decrease in the positive rate of Ki-67 was the most significant, from 75.9% before NAC to 41.1% after NAC (P<0.001). Studies by Makris et al., and Yin et al., showed a statistically significant decrease in Ki-67 proliferation index following NACT (P=0.001 and P=0.01, respectively). However, result of Wu et al.,[14] was not concordant with other studies. They reported Ki-67 conversion from positive to negative in 21.1% of the cases and negative to positive in 23.7% of the cases. According to him the decrease in the positive conversion was from 65% to 43.4% which was not significant.

In the study conducted by Tacca et al.,[11] 42% of cases that were initially HR negative became positive whereas 13% of cases that were HR-positive became negative. Overall change in HR status was observed in 23% of the cases post-NACT which was significant. Tacca et al.,[11] found patients with HR-negative tumors which switched to a positive status after NACT had better overall survival (OS) and disease-free survival (DFS) than patients whose tumor remained HR negative.

Hirata et al.,[12] verified patients whose HR status shifted from negative to positive after NACT, if administered endocrine therapy, had a better prognosis than patients who were HR negative before and after NAC.

We also found that the expression of ER, PR, and HER2/Neu are highly dependent on each other, modulation of one receptor can change the expression of another receptor as well. In our study, the change in the expression of ER was significantly associated with a change in the expression of PR and vice versa while NPI score was significantly associated with a change in HER2/Neu. No significant

association was seen between changes in any of the HR status with other clincopathological parameters.

Peng et al.,[13] while doing multivariate regression analysis observed that changes of markers were defined as the dependent variables. Lateral superior quadrant was observed to be independently associated with change in ER (negative→positive). Increased number of lymph nodes and body mass index seemed to be related to the conversion of PR (positive→negative). Moreover, there was a statistical association between the Ki-67 (positive→negative) and the age ≥50. Number of lymph nodes ≥1 and TNM stage 1–2 were statistically associated with changes in HER2 (positive→negative). All other tested variables were not associated with the conversion of markers.[13] According to Tan et al.,[17] a relatively high proportion of high Ki-67 indexes were observed in tumors with HR alteration compared to tumors in which HR status remained negative. Other clinicopathological features, such as age, menopausal status axillary node status, and tumor size were not associated with HR conversions significantly.[17]

Causes of change in receptor status in CNB and excision biopsy could be due to heterogeneity, laboratory procedure, or observer variability. The first reason could be technical. In a study conducted by Tacca et al.,[11] the discordance rate of HR status was evaluated in 100 patients not treated with NACT as a control group. The discordance rate observed was 3%; hence, they concluded that although some discordance might be caused by technical caveats but had minor clinical significance. The second reason could be due to differences in the primary and metastatic tumor of the same patient and the third reason could be changes induced by the treatment itself.[11] Tumor heterogeneity and the time interval between biopsy and surgery are other sources of bias.[13]

The possible mechanisms for change in receptor status in BCs caused by chemotherapy are complicated. Chemotherapy agents might directly or indirectly change the biology of tumor cells.

The following hypotheses have been proposed to explain the mechanism: According to Tan et al.,[17] NACT while targeting chemosensitive tumor cells may leave insensitive tumor cells with different biology as a residual disease. Tacca et al.,[11] explained HR status discordance by two hypotheses; first it could be a result of the selection of certain tumor clones during treatment with a selective disappearance of either HR-positive or HR-negative tumor cells because it is generally known that HR-negative tumors are more sensitive to chemotherapy than HR-positive tumors. Another explanation could be that receptors could be re-expressed in the tumor cells and a positive switch in HR could be the result of therapy-induced re-expression of HR on the nuclei of the tumor cells.[11]

Another important aspect is to assess the magnitude of the change of the Allred score and to determine its statistical significance.[11]

Whatever the reason, HR status switch has been significantly correlated with the overall survival and disease-free survival of the patients in the literature.[11-17]

In patients from positive to negative switch, Chen et al.,[18] have reported the benefits from endocrine therapy as compared to patients whose HR remains stable. In contrast, Tacca et al.,[11] and Hirata et al.,[12] observed no significant difference in overall survival and disease-free survival between two groups, that is, patients with HR switch and HR stable before and after NACT.[11]

Tan et al.,[17] significantly correlated positive HR status switch with better progression-free survival and overall survival in patients that were treated with adjuvant endocrine therapy; however, patients with a negative switch of HR status may benefit less with endocrine therapy compared to patients whose HR status remains positive.[17]

These findings in different studies indicate that a positive switch of HR status could be an indicator for a better outcome, while a negative switch seemed to be associated with a worse prognosis so it is necessary to determine the HR status before and after NAC and to administer endocrine therapy to patients with HR status conversion.

### Strength and limitations of the study

In concordance with previous studies, we did find significant changes in IHC expression of ER, PR, and Ki-67 in patients with BC who received NACT.

Despite yielding these clinically relevant findings, our study was limited in some aspects: The patient groups studied were heterogenous in terms of sample size and characteristics, the sample size was small and no assessment of OS and DFS could be done in our study as due to COVID pandemic, follow up of all the patients could not be done.

## CONCLUSION

Our observational study demonstrated the existence of discordance in the HR status and proliferation marker after NACT in patients with BC. The administration of NACT might be the main reason for the change in receptor status thus understanding the chemotherapy-induced biological conversion in the tumor cell behavior

is strategically important in planning adjuvant endocrine therapy and for disease follow-up. The biological effects of chemotherapeutic agents on cancer cells other than cell death need to be thoroughly investigated. In the future, further studies are required to identify the mechanism for this switch in receptor status after NAC and to validate the prognostic impact associated with this switch.

Furthermore, the significant switch in the HR status after NACT underlines the importance of taking into account the HR status of the residual tumor for eventual adjuvant endocrine therapy.

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