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Effect of Neo-Adjuvant Chemotherapy On Estrogen Receptor (ER), Progesterone (PR) Receptor, Human Epidermal Growth Factor Receptor 2 (HER2), and Ki-67 Index In Breast Carcinoma

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ABSTRACT

Objective: To know the outcome of neo-adjuvant therapy on hormone receptors, HER2, and Ki-67 index in invasive breast carcinoma.

Study Design: Prospective analytical study

Place and Duration of Study: Histopathology department, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from Dec 21 to Oct 22.

Methodology: We included 110 patients with histologically proven invasive breast carcinoma in our study. Rate of expression of ER, PR, HER2, and Ki-67 index was assessed in pre-neoadjuvant needle-core specimens, followed by administration of neo-adjuvant chemotherapy, and then the receptor statuses were evaluated on surgically excised specimens of the same patients. A paired t-test was applied to analyse the changes in receptor expression.

Results: Among 110 patients, the average age was 48.47±10.12 years. The most common histological type was invasive ductal carcinoma, no special type noted in 97 (88.2%), and the most common histological grade was II, noted in 66 (60%). Discordance of ER is noted in 34 (30.91%) cases, and for PR in 29 (26.37%) cases. Overall, HER2 receptor negativity increased from 41 (37.30%) to 51 (46.40%) cases. The average Ki-67 index decreased from 27.34% to 24.03% after administration of chemotherapy.

Conclusion: Neo-adjuvant chemotherapy administration may cause changes in receptor expression, mainly in ER and Ki-67 index. These findings are helpful in the choice of sequential adjuvant therapy and improve treatment and prognosis in invasive breast carcinoma.

Keywords: Estrogen Receptor, HER2, Invasive Breast Carcinoma, Ki-67 Index, Neo-Adjuvant Chemotherapy, Progesterone Receptor.

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INTRODUCTION

Breast cancer is the most commonly occurring cancer among women and the second most common cancer worldwide. According to a study based on demographic data, one in every nine women in Pakistan is at a lifetime risk of being diagnosed with breast cancer. Pakistan possesses the highest age-standardised incidence rate of breast cancer among all Asian countries. Most cases of breast cancer are reported among women in the postmenopausal period. With 60-64 years being the peak years.[1] Demographic data available on breast cancer cases points towards an alarming public health concern for Pakistan in the near future.

WHO, in its awareness campaigns, encourages prolonged breastfeeding, moderate physical activity, weight control, avoidance of alcohol, avoidance of radiation exposure, and the use of prolonged hormonal therapies to decrease the risk of breast cancer. Increased levels of androgens and progesterone are known to be associated with breast cancer.[2] Use of hair dye containing paraphenylenediamine, lack of awareness, and checking of personal items have also been associated with breast cancer in the past. Mutations in the gene, abnormal amplification of the oncogene, and vitamin D deficiency have also been suggested as possible causes of breast cancer. Studies have reported an association between high levels of androgens and estrogen and breast cancer.

Neo-adjuvant chemotherapy (NAC) is based on systemic treatment before surgery. Previously, the concept of neo-adjuvant chemotherapy was based on its use in patients who had advanced or inoperable breast cancer.[3] The main purpose in such patients was to downstage the cancer by reducing the size of the tumour. Patients who are not willing to undergo extensive surgery and want to omit dissection of axillary lymph nodes are good candidates for breast-conservation surgery after NAC. With advances in the

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field, the use of neo-adjuvant chemotherapy has been widened to improve aesthetic and cosmetic outcomes. With the use of NAC, postoperative complications such as lymphedema can also be avoided. In another study conducted by "The Early Breast Cancer Trialists Group," data were collected from 4756 women who were randomly assigned to receive either neo-adjuvant chemotherapy or adjuvant chemotherapy with a follow-up of 9 years. Neoadjuvant chemotherapy was found to have the highest rate of breast-conserving therapy as compared to adjuvant chemotherapy.[4]

Human epidermal growth factor receptor 2 (HER2) is one of the epidermal growth receptors. HER2 encodes the receptor tyrosine kinase and is overexpressed and amplified in breast cancer, and it is associated with more aggressive behaviour.[5] The upregulation of HER2 causes the progression of the tumour. Several levels and aspects of tumour growth are known to be reflected and mediated through signalling via HER2. With the use of HER-2 directed therapies among breast cancer patients, the outcome in HER2 positive breast cancer cases has been found promising.[6]

The involvement of ovarian hormones, oestrogen, progesterone, and their receptors (ER/PR) has been found in breast cancer development and proliferation.[7] With alteration of ER/PR receptor tumours, there are likely chances of chemo-resistance development. Expression of these receptors is a significant and powerful predictor in the treatment planning of breast cancer patients.[8]

Several studies have considered ER, PR, and HER2 status as important prognostic factors in breast cancer. This status plays a crucial role in the decision of adjuvant therapy after the patient has undergone surgery. Different studies have reported variable outcomes after administration of neo-adjuvant chemotherapy. ER, PR, and HER2 were among the first biomarkers of choice for routine clinical use. To date, they are known to hold position of a strong predictor of outcome after endocrine therapy. There is an indication of endocrine treatment in all patients with a positive hormone receptor status.[9] Neoadjuvant therapy has an impact on Estrogen receptor, progesterone receptor, HER2 receptor, and Ki-67 index in breast cancer patients, and the receptor status can be utilised as a prognostic factor to decide future course of treatment.

METHODOLOGY

This prospective analytical study was conducted at Histopathology Department, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from Dec 2021 to Oct 2022. This study recruited 110 cases, who were histologically diagnosed cases of invasive breast carcinoma on core-needle biopsy specimens. Sample size was calculated by using the WHO sample size calculator, keeping a confidence interval of 95%, a margin of error of 6%, and an anticipated frequency of breast cancer to be 11.7% in a previous study.[10,11]

After obtaining ERC from the Institutional Review Board under. A sample size of 111 was calculated, but we included 110 patients in our study. A non-probability convenience sampling technique was used. Inflammatory breast carcinoma, metastatic breast carcinoma, and in situ lesions were excluded from the study. Immunohistochemistry for ER, PR, HER2, and Ki-67 index expression was applied to 5 µm-thick sections on the block bearing the maximum tumour area. Staining was performed using an automated Leica Bond–III stainer. Positive and negative controls were included, and DAB (3,3' diaminobenzidine) was used as a chromogenic agent. Sections were assessed simultaneously by 2 histopathologists. ER, PR, and Ki-67 index expressions were noted as negative and positive based on nuclear staining in tumour cells according to the Allred Scoring System. Hormone receptors are regarded as positive when there is staining of >1% tumour cells, whereas HER2 is graded as: negative (0+) when there is no membranous staining of tumour cells, negative (1+) when there is incomplete faint membranous staining within >10% of tumour cells, intermediate (2+) when there is weak/faint circumferential membranous staining in >10% of tumour cells or complete membranous staining in <10% of tumour cells and positive (3+) when there is complete, strong, crisp membranous staining in >10% of tumour cells following guidelines of College of American Pathologists (CAP) and American Society of Clinical Oncology (ASCO). Ki-67 nuclear labelling index is labelled as low when there is ≤14% nuclear expression in tumour cells, and high when there is >14% nuclear expression in tumour cells.

The rate of change of expression of hormone receptors, HER2, and Ki-67 index in paired samples from needle-core incisional biopsy and surgical excision specimens after neo-adjuvant chemotherapy of the same patients using Statistical Package for Social

Sciences version 23.0 was evaluated. The chi-square test was applied to assess the association between the variables. The *p*-value of ≤ 0.05 was considered statistically significant.

RESULTS

110 patients were recruited in the study. During the study, all patients have undergone neo-adjuvant chemotherapy followed by breast surgery. Mean age was 48.47±10.12 years. Regarding laterality, 65(59.1%) patients presented with right-sided breast carcinoma, whereas 45(40.9%) patients presented with left-sided breast carcinoma. The most common histological type was invasive ductal carcinoma, no special type, 97 (88.2%), followed by invasive lobular carcinoma, comprising 11(10%) cases, and 2(1.8%) cases of micropapillary carcinoma were reported. Grading of the tumour was done by using the Nottingham histological grading score. The most common histological grade was grade II, 66(60%), followed by grade III, 36(32.7%), and grade I was the least common grade, 8(7.3%), as shown in Table-I.

Table-I: Characteristics of patients (n=110)

Variables	Values
Age (years)	48.47±10.17
Affected side, n (%)	
Left	45 (40.9%)
Right	65 (59.1%)
Histological type, n (%)	
Invasive ductal carcinoma, NST	97 (88.2%)
Invasive lobular carcinoma	11 (10.0%)
Micropapillary carcinoma	2 (1.8%)
Nottingham histological grade, n (%)	
Grade-I	8 (7.3%)
Grade-II	66 (60.0%)
Grade-III	36 (32.7%)

Our results depict discordance of estrogen receptor in 34(30.91%) of the recruited cases, as shown in Table II.

Table-II: Changes of estrogen receptor (ER) before and after neo-adjuvant chemotherapy administration (n=110)

Estrogen Receptor (ER)	Post chemotherapy		Changes Turnover number (%)	*p*-Value
Prechemotherapy	Positive (n=63)	Negative (n=47)		
Positive (n=83)	56(50.91%)	27(24.55%)	30.91	<0.001
Negative (n=27)	7(6.36%)	20(18.18%)		

However, in the case of progesterone receptors, a scarcity of discordance was noted, and the majority of cases maintained the same receptor status as before administration of chemotherapy, as shown in Table III. Discordance was noted among HER2 receptors. Before chemotherapy, 45(40.9%) receptors were noted as positive for HER2 (3+), which changed to 31(28.18%) following the administration of chemotherapy, as shown in Table IV. The average index of Ki-67 decreased from 27.34% to 24.03%.

Table-III: Changes of progesterone receptor (PR) before and after neo-adjuvant chemotherapy administration (n=110)

Progesterone Receptor (PR)	Post chemotherapy		Changes Turnover number (%)	*p*-value
Prechemotherapy	Positive (n=100)	Negative (n=10)		
Positive (n=75)	73(66.36%)	2(1.82%)	26.37	0.057
Negative (n=35)	27(24.55%)	8(7.27%)		

Table-IV: Changes of HER2 before and after neo-adjuvant chemotherapy administration (n=110)

Grade	Post-chemotherapy			*p*-value
Pre-chemotherapy	Positive (n=31)	Intermediate (n=28)	Negative (n=10)	
Positive (n=45)	31 (28.18%)	4 (3.64%)	10 (9.09%)	<0.001
Intermediate (n=24)	0 (0.00%)	24 (21.80%)	0 (0%)	
Negative (n=41)	0 (0.00%)	0 (0.00%)	41 (37.30%)	

DISCUSSION

Breast carcinoma is the most common malignancy among females worldwide. The most significant finding of our study is a change in hormone receptor status following chemotherapy. Our results show discordance of the estrogen receptor in a significant percentage of patients. Using a paired sample t-test, a significant difference between the means of both groups was noted, i.e., *p*-value < 0.05. The progesterone receptor retained the same status as before chemotherapy, as the comparison between both means was deemed statistically insignificant, i.e., *p*-value > 0.05. Discordance was also observed in HER2 receptors. The decrease in HER2 positivity was statistically significant, i.e., *p*-value < 0.05. The difference between the means of the pre- and post-chemotherapy groups for the Ki-67 index was statistically significant, i.e., *p*-value < 0.05.

In Pakistan, due to a lack of awareness and screening programs, cases are mostly diagnosed at an advanced stage, leading to a poor prognosis. Patients usually prefer non-invasive and cosmetically acceptable treatment options like neoadjuvant chemotherapy and radiotherapy.[12] Neo-adjuvant chemotherapy reduces the tumour burden and treats

micrometastatic lesions. Administration of neo-adjuvant chemotherapy alters the hormone receptors and HER2 receptor, thus hormone receptors, HER2, and Ki-67 index should be re-checked to confirm their receptor status and thus help in the choice of treatment modalities.[13]

Literature has reported variable results related to alteration in receptor status following neoadjuvant chemotherapy. A study conducted by Abu El-Ela *et al.*, reported in a tertiary care setting showed 17% discordance in ER, 13% discordance in PR, and 11% discordance with HER2.[14] Zhu *et al.*, reported a decrease in the positivity of receptors after neoadjuvant chemotherapy.[15] Roskoski *et al.*, showed 29% discordance in receptor status.16 Hu *et al.*, showed a 42 percent change from negative to positive.[17] This study involved 26 patients, who showed an increase in positivity of HER2 by 23%. A retrospective trial of 87 cases depicts a 15% change in HER2 expression. A prospective trial of 73 cases conducted by Kawsar *et al.*, revealed a 15% change in HER2 expression, but no switch was noted on fluorescence in situ hybridization.[18] A decrease in the positivity of the Ki-67 index from 75.9% to 41.1% was analyzed, highlighting a significant decrease. Peng *et al.*, noted a downhill trend in the expression of the Ki-67 index. Thus, supporting results of the present study.[19]

However, literature review enlightens us about several studies giving results contradictory to ours. The retrospective study conducted by Badr et al., highlighted the role of the effect on ER and PR expression, revealing no change in ER and PR status following chemotherapy.[20] Another trial conducted by Schettini et al., revealed no change in hormone expression.[21] No significant change in expression of ER, PR, and HER2. Literature reveals some contrast in results on the immunohistochemistry and FISH techniques. de Mooij *et al.*, reported a 35% change in the status of HER2 on immunomarker studies, whereas a 13% transition was observed on the fluorescence in situ hybridization technique. Additionally, the study also showed a 2.3% change in receptor status on immunohistochemistry but no change on FISH technique. Similar percentage of 4.8% on both immunohistochemical markers as well as FISH study. A 20% change in receptor status on immunohistochemical markers and no alteration in expression of HER2.[22]

LIMITATION OF STUDY

FISH for HER2 could also be considered a limitation of the present study and a recommendation for future studies to confirm HER2 receptor status. Moreover, considering the prevalence of invasive breast carcinoma, the sample size of the present study was limited.

CONCLUSION

Changes in receptor status of ER, PR, and HER2 following administration of neo-adjuvant chemotherapy have been reported. Studies have suggested several possible factors involved in change in receptor expression following neo-adjuvant chemotherapy, such as chemo-resistant clones, tumour heterogeneity, ovarian suppression, change in gene expression, fixation, and staining techniques. In the future, thorough studies are required to enlighten us about the exact pathophysiology behind the alteration in receptor status to confirm the prognostic effect associated with this alteration.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

WAK & MA: Data acquisition, data analysis, critical review, approval of the final version to be published.

HUD & NZ: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

FR & HT & UA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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