Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.304607.3478 Volume 30, Issue 1.7, Oct. 2024, Supplement Issue
Manuscript ID: ZUMJ-2407-3478
DOI: 10.21608/zumj.2024.304607.3478.

ORIGINAL ARTICLE

The Effect of Neoadjuvant Chemotherapy on Her-2 Status in Her-2 Negative Breast Cancer Patients: A Study on Pathological Response

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Submit Date: 23-07-2024
Revise Date: 20-08-2024
Accept Date: 24-08-2024

ABSTRACT

Background: The impact of neoadjuvant chemotherapy (NAC) on Her-2 status in low Her-2 breast cancer (BC) is not well understood. We assessed the change of Her-2 status in Her2- low and her-2 score 0 in BC patients following NAC. **Methods:** A retrospective study involved a total 469 patients with non-metastatic breast cancer managed in private hospitals in the Republic of the Sudan. **Results:** A total of 469 patients were eligible and included. Post NAC, 250 (53.3%) patients had a Her-2 score of 0 (176 had unchangeable Her-2 score of 0 and 74 were changed from low Her-2 to Her-2 score 0), while 219 (46.7%) had a low Her-2 (151 had unchangeable low Her-2 and 68 were changed from Her-2 score 0 to low Her-2). Patients with unchanged low Her-2 or changed from low Her-2 score zero had a higher percentage of ER positivity (65.6% and 58.1%) than those with changes from Her-2 score 0 to low (61.7%) or with unchanged Her-2 score 0 (52.8%), respectively. Patients with low Her-2 post-NAC who maintained low Her-2 had higher grade, Ki-67, and ER+ status compared to those whose Her-2 status changed. Patients with a Her-2 score of 0 post-NAC had higher Ki-67 levels and were more likely to be ER+. There was no significant correlation between post-NAC changes in Her-2 status and RCB. **Conclusion**: Changes in low Her2- expression were observed in post-NAC BC patients. Further investigation is needed to recognize the prognostic values of Her-2 changes in low Her-2-BC.

Keywords: Low Her-2, Her-2 score 0, breast cancer, neoadjuvant chemotherapy.

INTRODUCTION

Breast cancer (BC) is a complex and diverse disease with various subtypes determined by the levels of estrogen receptor (ER), progesterone receptor (PR), Human epidermal growth factor receptor 2 (Her-2), and Ki-67 expression. While genetic research has advanced, treatment decisions continue to rely heavily on assessing ER, PR, Her-2, and Ki-67 [1]. New anti-HER2 antibody-drug conjugates (ADCs) like trastuzumab deruxtecan (T-DXd) are showing effectiveness in Her2-low BC patients. This has led to further research on the clinical and biology significance of patients with Her2-low BC. Her-2 low refers to tumors with an immunohistochemistry (IHC) score of 1+ or 2+ and negative in situ hybridization (ISH) [2].

Neoadjuvant treatment (NAC) is the standard of care in many subtypes of BC including locally tumor size >= T2, involvement of axillary lymph nodes, inflammatory breast cancer, Her-2 +ve, and triple negative breast cancer (TNBC) [3]. NAC is increasingly used to improve complete pathological response (pCR) in BC, which is a key predictor of survival outcomes. Numerous studies have shown that pCR is a crucial prognostic factor after NAC. Attaining pCR post-surgery is associated with a significant enhancement in disease-free survival (DFS) [1]. Many data regarding the ability of NAC to affect the

expression of molecular markers of BC with wide variation in the results, ER changes up to 23% and PR up to 63%. HER2 status changes less frequently [4].

Changes in Her2 status, from positive to negative or vice versa in residual tumors after NAC have been commonly observed. The impact of these changes on prognosis has been studied. However, transitions involving Her2-low status, such as Her2-score 0 to low or positive, have not been clearly defined [5].

This study aims to investigate the changes in Her-2 status following NAC in patient with low Her-2 expression and Her-2 score 0 and correlation with pathological response.

METHODS

Sample Size

A retrospective study involved a total 469 patients with BC, non-metastatic patients included the study managed in private hospitals in the Republic of the Sudan during the period from February 2016 to December 2021.

Eligibility Criteria

Patients aged 18 years or older with a confirmed diagnosis of BC, low Her-2 expression or Her-2 score 0, Eastern Cooperative Oncology Group (ECOG) Performance Status of 0-2, no prior chemotherapy or radiotherapy, measurable disease, normal organ function, available formalin-fixed and/or paraffin-embedded tissue samples, non-complete pathological response (non-pCR) and sufficient follow-up data were included in the study.

Data Collection

The clinical data, including demographic and pathological information, is reviewed and recorded in the patients' chart files.

Immunohistochemistry

The IHC evaluation were conducted before initiating NAC using a tru-cut biopsy. Another evaluation was done after completing the surgical management, which may involve either breast-conserving surgery (BCS) or modified radical mastectomy (MRM).

Hormone receptor positive is defined as less than 1% of cells showing staining for ER and PR expression. Low Her-2 refers to tumors with an IHC score of 1+ or 2+ and negative in situ hybridization (ISH), while a Her-2 score of 0 indicates no staining [6]. A Ki-67 level greater than 15% is classified as high, while 15% or less is considered low.

Chemotherapy Treatment Protocol

After a routine evaluation of organ reservoirs, including renal, hepatic, cardiac, and a complete blood count (CBC), the patient was admitted to the institution. Following the completion of the preplanned protocol, a metastatic workup was performed. If no metastases were found, the patient was then transitioned to surgical management.

Response Evaluation

Pathological evaluation will determine the presence of tumor residue in lymph nodes and breast tissues, which will be categorized as Residual Cancer Burden (RCB) levels: RCB-0 (complete pathological response), RCB-I (minimal residual disease), RCB-II (moderate residual disease), and RCB-III (extensive residual disease) [7]. Figure 1 shows the flow chart of the 837 BC patients included in the study.

The intervention used poses more than minimal harm to participants, so informed consent was not sought. Patient names are kept private and confidential in a pass code dataset, connected solely to a study ID. The study was approved by the institutional review board (IRB).

Statistical Analysis

The analysis was conducted using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). A significance level of $P < 0.05$ was used to determine statistical significance.

RESULTS

A total of 469 patients were eligible and included in the final analysis. Post NAC, 250 (53.3%) patients had a Her-2 score of 0 (176 had unchangeable Her-2 score of 0 and 74 were changed from low Her-2 to Her-2 score 0), while 219 (46.7%) had a low Her-2 (151 had unchangeable low Her-2 and 68 were changed from Her-2 score 0 to low Her-2).

Through the patients with low Her-2 post-NAC, those with unchanged low Her-2 compared to those with changed low Her-2 (from score 0 to low Her-2) showed higher grade, Ki-67, and ER+ status, which were statistically significant (p-values of 0.03, 0.01, and 0.04, respectively). The same trend was observed with PR+ status, although it was not statistically significant.

In all groups, there was no statistically significant correlation between post-NAC changes in Her-2 status and RCB (p-values were 0.4 and 0.8) (Table1). There was a decrease in ER+ tumor tissues in the low Her-2 group, whether the Her-2 changed from 0 to low Her-2 (62.9% to 58.8%) In contrast, ER+ showed an increase in positivity in cases with a Her-2 score of 0, whether changed or unchanged (60.2% to 64.9%).
Regarding the Ki-67 expression, marked reduction in high K-i67 post NAC compared to pretreatment (Table2). Figures (2-7) illustrate the different molecular features involved in the study.

Table (1): Main initial patient features based on Her-2 status.

	Her-2 low post NAC N (219)		**P value**	**Her-2 score 0 post NAC N (250)**		**P value**
Variables Pre NAC	**Unchanged low N (151)**	**Changed to low (68)**		**Unchanged Score 0(176)**	**Changed to score 0(74)**	
Age Median Range	47 (29-73)	46 (28-71)	0.3	46 (26-70)	46 (26-72)	0.5
Menopause status Premenopausal Postmenopausal	 60(39.7%) 91(60.3%)	 43(63.2%) 25(36.8%)	0.1	 89(50.6%) 87(49.4%)	 32(43.2%) 42(56.8%)	0.8
Pathology IDC Non-IDC	 139(92.1%) 12(7.9%)	 61(89.7%) 7(10.3%)	0.7	 161(91.5%) 15(8.5%)	 64(91.9%) 10(8.1%)	0.2
Grade I II II	 23(15.2%) 54(35.8%) 74(49.0%)	 15(22.1%) 32(47.1%) 21(30.8%)	 0.03	 31(17.6%) 49(27.8%) 96(54.6%)	 17(23.0%) 21(28.4%) 36(48.6%)	 0.07
LVI Yes No	 69(45.7%) 82(54.3%)	 41(60.3%) 27(39.7%)	 1.00	 86(48.9%) 90(51.1%)	 39(52.7%) 35(47.3%)	 0.9
T size T1 T2 T3 T4	 21(13.9%) 63(41.7%) 42(27.8%) 25(16.6%)	 6(8.8%) 31(45.6%) 22(32.4%) 9(13.2%)	 0.7	 30(17.0%) 75(42.6%) 51(29.0%) 20(11.4%)	 6(8.1%) 23(31.1%) 27(36.5%) 18(24.3%)	 1.00

NAC, neoadjuvant chemotherapy; IDC, invasive duct carcinoma; LVI, lymph-vascular invasion; ER, Estrogen Receptor; PR, progesterone Receptor; P value < .05 is significant.

Table (2): The correlation between Her-2 Status, Ki-67, ER+, PR+, and pathological response of NAC

	Her-2 low post NAC N (219)		P value	Her-2 score 0 post NAC N (250)		P value
Variables Post NAC	**Unchanged low N (151)**	**Changed to low (68)**		**Unchanged Score 0(176)**	**Changed to score 0(74)**	
Ki-67						
Low	80(53.0%)	37(54.4%)	0.01	89(50.6%)	41(55.4%)	0.03
High	71(47.0%)	31(45.6%)		87(49.4%)	33(44.6%)	
ER +ve	95(62.9%)	40(58.8%)	0.08	106(60.2%)	48(64.9%)	0.002
PR+ve	61(40.4%)	39(57.4%)	0.9	73(41.5%)	29(39.2%)	1.0
Non- RCB Class						
I	37(24.5%)	11(16.2%)		39(22.2%)	13(17.6%)	
II	61(40.4%)	42(61.8%)	0.4	72(40.9%)	39(52.7%)	0.8
III	53(35.1%)	15(22%)		65(36.9%)	22(29.7%)	

NAC, neoadjuvant chemotherapy, ER, Estrogen Receptor; PR, Progesterone Receptor; RCB, Residual Cancer Burden.

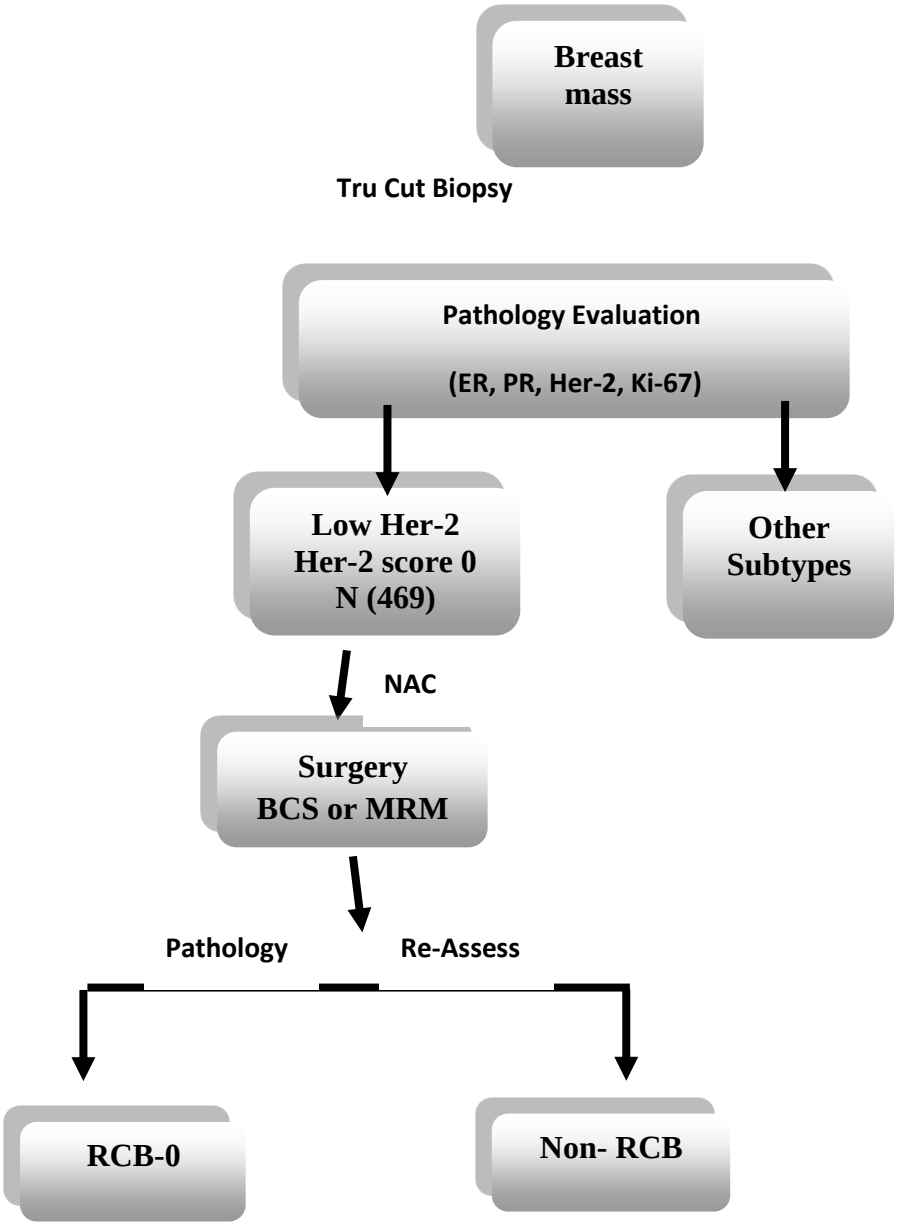

Figure (1) shows the flow chart of BC patients included in the study.
Her-2 low refers to tumors with an immunohistochemistry score of 1+ or 2+ and negative in situ hybridization; NAC, neoadjuvant chemotherapy; BCS, breast conserving surgery; MRM, modified radical mastectomy; RCB, Residual Cancer Burden; RCB-0, complete pathological response.

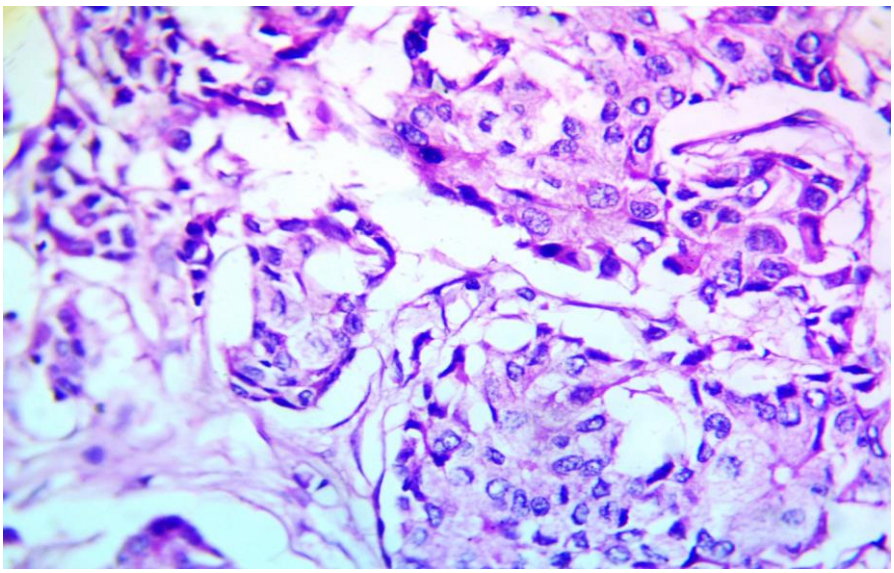

Figure (2) shows high-grade invasive ductal carcinoma on H&E staining at 400x magnification.

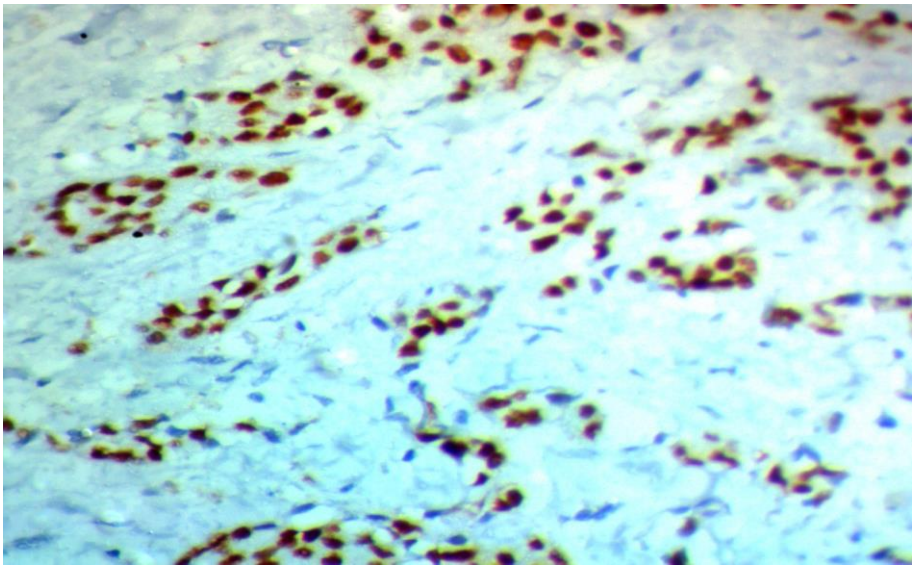

Figure (3) shows strong and diffuse ER nuclear staining on immunohistochemistry at 400x magnification.

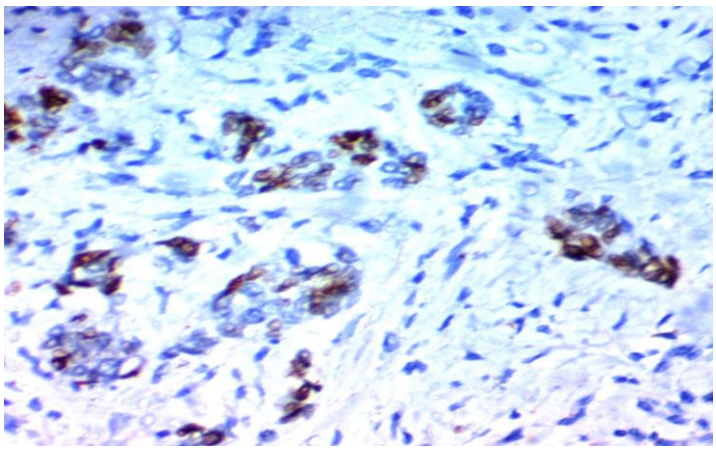

Figure (4) shows moderate PR nuclear staining on immunohistochemistry at 400x magnification.

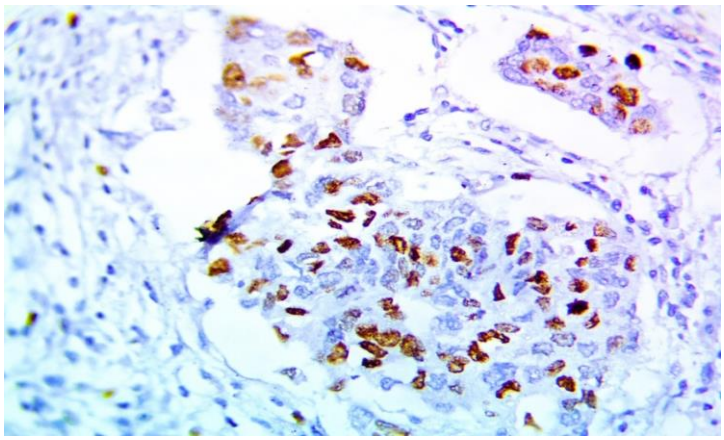

Figure (5) shows a high proliferation index with Ki67 > 14% on immunohistochemistry at 400x magnification.

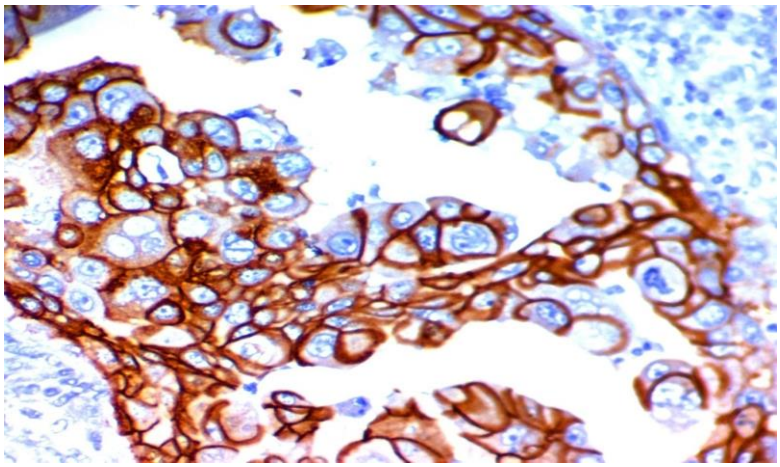

Figure (6) shows strong membranous expression of HER2/neu in more than 10% of malignant cells (score 3) on immunohistochemistry at 400x magnification.

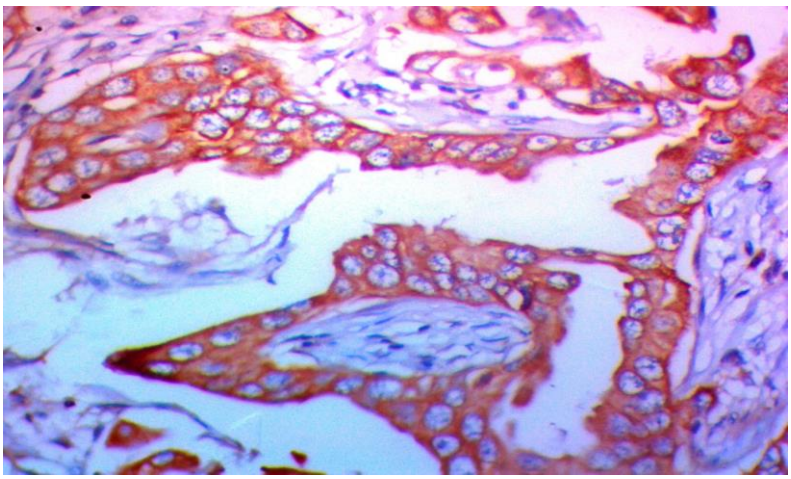

Figure (7) shows strong membranous expression of HER2/neu in more than 10% of malignant cells (score 2) on immunohistochemistry at 400x magnification.

DISCUSSION

NAC can affect tumor gene expression, potentially changing the IHC biomarker profile. BC studies have shown tumor heterogeneity, with varying ER and Her-2 expression within individual tumors. This heterogeneity can impact patient outcomes. The current study assessed changes in low Her2 expression in patients who received NAC followed by surgery. Patients with unchanged low Her-2 or changed to Her-2 score 0 had higher ER +ve rates (65% and 58.1%, respectively) compared to those transitioning from Her2-score 0 to low (61.7%) or remaining Her2-score 0 (52.8%). These findings suggest a strong association between Her2-low and ER +ve. The study confirms previous reports of Her2-low instability post-NAC and its link to ER +ve status [8, 9].

Two studies reported that the instability of Her-2 expression was more pronounced in ER + subgroups compared to ER- subgroups [10, 11]. The reasons behind the fluctuating Her-2 expression levels post-NAC are not fully understood. A plausible hypothesis is that chemotherapy-induced selective pressure may lead to changes in Her-2 expression levels as a response to treatment resistance. Moreover, discrepancies in Her2-low expression among pathologists could be attributed to the lack of clear guidelines for differentiating between lower levels of Her-2 expression, especially between IHC0 and IHC1+. It is expected that this issue will be clarified by the Destiny-Breast 06 study, which involves central evaluations of Her-2 status. It is essential to develop precise Her-2 testing techniques to effectively identify lower expression levels of Her2 in the future [11-13].

Few studies have explored the prognostic implications of Her-2 status changes post-neoadjuvant NAC. They found no significant association between changes in Her-2 status and DFS compared to patients with unchanged Her-2 status. However, findings from the German Breast Group (GBG) at the 2022 suggest that transitioning from Her-2-negative to Her-2-low is linked to reduce invasive DFS in ER+ tumor patients after NAC. This shift was associated with a higher risk of recurrence compared to patients with consistently low Her-2 status [10, 14]. The varying results may be due to differences in patient features. Miglietta *et al*. analyzed the all patients without considering ER status, while the GBG study focused on ER+ tumors. Miglietta *et al*. compared cases with initial Her2-low or Her2-score 0, while the GBG study concentrated at post-therapeutic Her2-low patients. Due to these differences and conflicting results, comparing the studies and coming to a firm

conclusion about the prognostic significance of dynamic changes in Her2-low expression following NAC is challenging.

Our study observes gain in ER status in either Her-2 score 0 patients (60.2% and 64.9%, post NAC). Chemotherapy can impact tumor biology and alter biomarker expression. Loss or decrease of ER expression has been linked to poorer tumor features and clinical behaviors. It may indicate reduce response to endocrine therapy [15-18]. Loss of ER expression is linked to poor clinical outcomes and affects response to endocrine therapy. Conversely, gaining hormone receptor expression after NAC is associated with better outcomes. However, such gains are infrequent [19, 20].

NAC treatment targets proliferation pathways and cycling cells, leading to a decrease in the size of tumor and Ki-67 expression. Reduction in Ki-67 expression post-NAC is linked with better clinical outcomes, including overall and disease-free survival. Studies suggest that post-NAT Ki67 levels are more predictive of survival than baseline levels [21, 22].

Now Her2-low is a predictive biomarker for the efficacy of T-DXd in metastatic BC. T-DXd is being considered for NAC. In low Her-2, no anti-Her-2 antibodies are approved for adjuvant setting. Better adjuvant chemotherapy is needed for Her2-low BC. Future trials should explore the use of anti-Her-2 antibody drug conjugate (ADCs). Her-2 expression can change between residual disease and primary. Testing Her-2 status in both may aid identify suitable candidates for adjuvant treatment with anti-Her-2 ADCs.

Our results offer valuable insights, definitive conclusions are limited by the current evidence. Further research is needed to clarify the prognostic significance of Her-2-low transition post-NAC. Our findings will inform future studies in this area.

Limitations:

The following study had some limitations. It was a retrospective, single-centre, and small sample size study. There was no central review of Her-2 expression. We did not assess molecular status and genomic profiles, which could provide insights into the Her-2-low biology. Furthermore, we did not analyze variations in outcomes based on these modifications. Additionally, our study only involved local patients, so the results may not be generalizable to all populations due to ethnic disparities in BC characteristics and outcomes.

CONCLUSION

NAC has been shown to have an impact on HER-2 status in patients with HER-2 negative BC either Her-2 low or score 0. Also, our findings showed the significant changes in Ki-67 after NAC, which can be of value in assessment patient outcomes. The results of this study will provide valuable insights into the effects of NAC on HER-2 status and may have implications for treatment decisions in these subtypes of patients. However, the classification of HER2-low BC as a distinct entity is still a matter of debate, with conflicting evidence regarding its prognostic significance in BC.

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ALI, E. The Effect of Neoadjuvant Chemotherapy on Her-2 Status in Her-2 Negative Breast Cancer Patients: A Study on Pathological Response. *Zagazig University Medical Journal*, 2024; (3977-3985): -. doi: 10.21608/zumj.2024.304607.3478