*Research Article*

# Change of estrogen receptor, progesterone receptor, and Ki-67 after neoadjuvant chemotherapy in patients with locally advanced breast cancer

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## Abstract

**Purpose:** The aim of this study is aimed to demonstrate the changes in the estrogen receptor (ER), progesterone receptor (PR), Ki-67, after neoadjuvant chemotherapy in the patients with locally advanced breast cancer. **Materials and Methods:** seventy patients who diagnosed with locally advanced breast cancer and treated with neoadjuvant chemotherapy. Ki-67, ER, PR, tested by immunohistochemistry (IHC) was evaluated before and after neoadjuvant chemotherapy. **Results:** There was a statistically significant reduction in Ki-67, but no statistically significant reduction in ER, PR, after neoadjuvant chemotherapy. **Conclusion:** The significant change in the Ki-67 proliferation index may suggest the reduced proliferative activity of malignant cells with neoadjuvant chemotherapy.

**Keywords:** estrogen receptor, progesterone receptor**,** neoadjuvant chemotherapy

## Introduction

Breast cancer is considered a systemic disease; chemotherapy and radiotherapy are added to the treatment, in addition to surgical treatment. Despite the advances in diagnosis, the locally advanced breast cancer cases are common, especially in underdeveloped countries1-4. Neoadjuvant chemotherapy (NAC) administered in locally advanced breast cancer (LABC) improves the chance of breast-conserving surgery (BCS) and operability in the inoperable cancers4-9.

Surgery should be performed including the primary tumor bed in patients who will undergo breast conserving surgery after the NAC. Modified radical mastectomy after NAC is the main surgical method in inoperable cases and inflammatory breast cancer. Post NAC sentinel lymph node biopsy has been applied in recent years. All patients who complete NAC therapy should be given radiotherapy in the postoperative period regardless of NAC response2-10. 5 years of disease-free and overall survival with additional treatment modalities for multimodal NAC in LABC, is 84% in stage III A and ranges between 35-50% in stage III B2-10. The aim of this study is to evaluate the changes in Ki-67, estrogen receptor (ER), and progesterone receptor (PR) in patients under NAC due to LABC.

Because the LABC patient group is a very heterogeneous disease group, the types of treatment vary widely. NAC regimens containing many regimens until the maximum tumor response is achieved. Treatment response should be evaluated clinically and radiologically prior to surgery and pathologically after surgery1-9.

## Patients and methods

### ***Ethics***

This study was conducted at Minia university hospital and Minia Oncology Center. All patients were informed of the investigational nature of this study and provided their written informed consent.

### ***Patient background***

A total of 70 patients with stage II and III non metastatic primary infiltrating ductal

breast cancer that were treated with NAC at period from January 2015 till May 2016 follow up period extended till May 2019 was done. Tumor staging were stratified based on the TNM Classification of Malignant Tumors, The Union for International Cancer Control Seventh Edition.[11]

Tumors were classified into subtypes according to the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), HER2 and Ki-67.

Clinical evaluation included physical examination, blood tests, chest X-ray, mammography, ultrasound breast exam, breast magnetic resonance imaging (MRI) and core biopsy.
In locally advanced tumors (defined as cT3N1, cN2-3 or cT4), bone scintigraphy and body computed tomography were added to the staging workup. For chemotherapy response evaluation, dynamic breast MRI was performed prior to surgery.

***Pathology assessment***
Pre-treatment estrogen (ER) and progesterone receptors (PR) status was assessed by immunohistochemistry (IHC), and HER2 status was assessed by either fluorescent in situ hybridization (SICH) or a validated IHC method. For ER and PR, cases were considered as negative when the percentage of immunoreactive tumor cells was below 1%; the rest of the cases (≥1% of tumor cells stained) were classified as positive. For HER2, cases were considered positive if Herceptest result was 3+ and/or FISH showed a ratio HER2/CEP17 ≥ 2; the rest of the cases were classified as negative. Ki67 proliferation index was calculated and 14% was taken as a cutoff point. pCR was defined as the absence of invasive carcinoma both in the breast and the axilla, regardless of the presence of carcinoma in situ (ypT0/Tis ypN0).

***Neoadjuvant therapy regimen and surgery***
All patients received a TC protocol consisting of six courses of Docetaxel (75 mg/m2) and (600 mg/m2) cyclophosphamide every 3 weeks, [12,13]

***Clinical end points***
Therapeutic antitumour effects were assessed according to the Response Evaluation Criteria in Solid Tumors criteria.[14] The pCR was defined as the complete disappearance of the invasive compartment of the lesion with or without intraductal components, including the lymph nodes.[15]

DFS was defined as the time from surgery to death, locoregional recurrence or distant recurrence.
Patients for whom none of these events were recorded were censored at the date of their last known contact. 16

***Statistical method:***
The collected data were coded, tabulated, and statistically analyzed using **SPSS program (Statistical Package for Social Sciences) software version 25.**

Descriptive statistics were done for parametric quantitative data by mean ± standard deviation and for non-parametric quantitative data by median and inter-quartile range (IQR), while they were done for categorical data by number and percentage.

Distribution of the data was done by **Kolomogorov Smirnov test.**

Analyses were done for non-parametric quantitative data using **wilcoxon signed rank test** between the two times.
Analyses were done for qualitative data using **Chi square test** (expected number per cell > 5) and **Fisher's exact test** (expected number per cell < 5).
Survival analysis done using **Kaplan Meier** analysis
The level of significance was taken at (**P value < 0.05**)

## Results
The clinicopathological characteristics of the 70 breast cancer patients were recorded as shown in [Table 1]. The patient's age ranged from 27 to 70 years, 45(64.3%) were premenopausal and 23(35.7%) were postmenopausal. All patients were invasive duct carcinoma. Patients with grade II carcinoma were 92% while grade III carcinoma was 8%. Patients mostly were of

clinical tumor size T2 31.4%, 48.6% of patients were of T3, and 18.6% were of T4. Patients with positive axillary LN metastasis were 71.4%. ER was positive in 68.6%, PR was positive in 55.7%, and Her2 was positive in 18.7%, and Ki-67 was 14 or more in 82% of patients.

Ten out of seventy patients achieved pCR (14.3%), 5 patients were TNBC, 4 patients were HER2 and one patient was Luminal B.

**Table (1): showing clinicopathological characteristics.**

| | | N=70 |
|---|---|---|
| **Age** | **Range** | (27-70) |
| | **Mean ± SD** | 46.4±10.6 |
| **Age group** | **Premenopausal** | 45(64.3%) |
| | **Postmenopausal** | 25(35.7%) |
| **Molecular Class.** | **Her 2** | 13(18.6%) |
| | **TNBC** | 15(21.4%) |
| | **Lu A** | 10(14.3%) |
| | **Lu B** | 32(45.7%) |
| **Survival** | **Alive** | 43(61.4%) |
| | **Dead** | 27(38.6%) |
| **Disease free survival** | **Range** | (8-178) |
| | **Mean ± SD** | 115.3±51.7 |
| | **Median / IQR** | 130.2 / (69.4-169.3) |
| **T** | **T2** | 22(31.4%) |
| | **T3** | 34(48.6%) |
| | **T4** | 13(18.6%) |
| **N** | **N0** | 20(28.6%) |
| | **N1** | 48(68.6%) |
| | **N2** | 0(0%) |
| | **N3** | 2(2.9%) |
| **ER** | **+ve** | 48(68.6%) |
| | **-ve** | 22(31.4%) |
| **PR** | **+ve** | 39(55.7%) |
| | **-ve** | 31(44.3%) |
| **Local Treatment** | **Mastectomy** | 52(74.2%) |
| | **Conservative surgery** | 18(25.7%) |
| **KI 67** | **Low** | **9(12.9%)** |
| | **High** | **61(87.1%)** |

Before NAC tumor size was T2 (31.4%), T3 (48.6%), T4 (18.6%), after NAC 10 cases achieved pCR, T1 (15.7%), T2 (68.6%) with **p value <0.001.**

Before NAC patients with lymph node +ve were (71.4%), after NAC were (17.1%) and patients with lymph node –ve were (82.9%) with **p value <0.001.**

Before NAC patients with ER +ve were (68.6%), after NAC was (75.7%) with change in 3 patients changed from +ve to –ve, 7 patients changed from –ve to +ve which was not statistical significant.

Before NAC patients with PR +ve were (55.7%), after NAC was (62.9%) with change in 4 patients from +ve to –ve and 6 patients changed from –ve to +ve which was not statistical significant.

KI67 was ranging from (2-95) pre NAC with mean±SD 40.6±21.4 and after NAC was ranging from (2-80) with mean±SD 29.7±18.3 which was statistical significant (p value <0.001).

Before NAC patients with high KI67 were 61(87.1%), only 3 patients reached to low level < 14 but 27(38.6%) patients achieved reduction in KI67 value post NAC but not reached <14.

The Pre and Post NAC results are summarized in table 2.

**Table (2): Changes of the variables before and after NAC (n: 70)**

| | | Pre | Post | P value |
|---|---|---|---|---|
| | | N=70 | N=70 | |
| **T** | **T1** | 1(1.4%) | 11(15.7%) | ***<0.001**** |
| | **T2** | 22(31.4%) | 48(68.6%) | |
| | **T3** | 34(48.6%) | 0(0%) | |
| | **T4** | 13(18.6%) | 0(0%) | |
| **N** | **-Ve** | 20(28.6%) | 58(82.9%) | ***<0.001**** |
| | **+Ve** | 50(71.4%) | 12(17.1%) | |
| **ER** | **-Ve** | 22(31.4%) | 17(24.3%) | *0.132* |
| | **+Ve** | 48(68.6%) | 53(75.7%) | |
| **PR** | **-Ve** | 31(44.3%) | 26(37.1%) | *0.132* |
| | **+Ve** | 39(55.7%) | 44(62.9%) | |
| **KI 67** | **Range** | (2-95) | (2-80) | ***<0.001**** |
| | **Mean ± SD** | 40.6±21.4 | 29.7±18.3 | |
| | **Median / IQR** | 40 /(25-60) | 27.5 / (15-40) | |
| **KI 67** | **Low** | 9(12.9%) | 12(17.7%) | *0.083* |
| | **High** | 61(87.1%) | 58(82.9%) | |

***KI67 evaluation:***

***Predictive role of KI67:***

After NAC 27(38.6%) patients achieved reduction in ki67 but did not reached to lower limits of ki67<14, 10(100%) 0f them underwent pCR, this identified in table 3.

**Table 3: shows correlation between reduction in ki67 post NAC and pCR.**

| | | PCR | | P value |
|---|---|---|---|---|
| | | -Ve | +Ve | |
| | | N=60 | N=10 | |
| **KI change** | *Stationary* | 43(71.7%) | 0(82.9%) | ***<0.001**** |
| | *Decreased* | 17(28.3%) | 10(100%) | |

***Prognostic value of KI67***

At a median follow-up of 36 months, 30 recurrences (42.9%) were observed after NAC and curative surgery.

Patients with low levels of ki67 had better outcome and prolonged DFS from those with high levels of ki67 post NAC (P value 0.018), this identified and illustrated in table 4 and figure 1.

**Table 4: shows relation between KI67 post NAC and DFS.**

| Means for Survival Time | | | | | Overall comparisons | |
|---|---|---|---|---|---|---|
| | | | 95% CI | | | |
| ***KI post*** | **Estimate** | **Std. Error** | **Lower Bound** | **Upper Bound** | **$X^2$** | **P value** |
| **Low** | 171.8 | 6 | 160 | 183.6 | 5.56 | 0.018* |
| **High** | 125.3 | 8.1 | 109.4 | 141.2 | | |

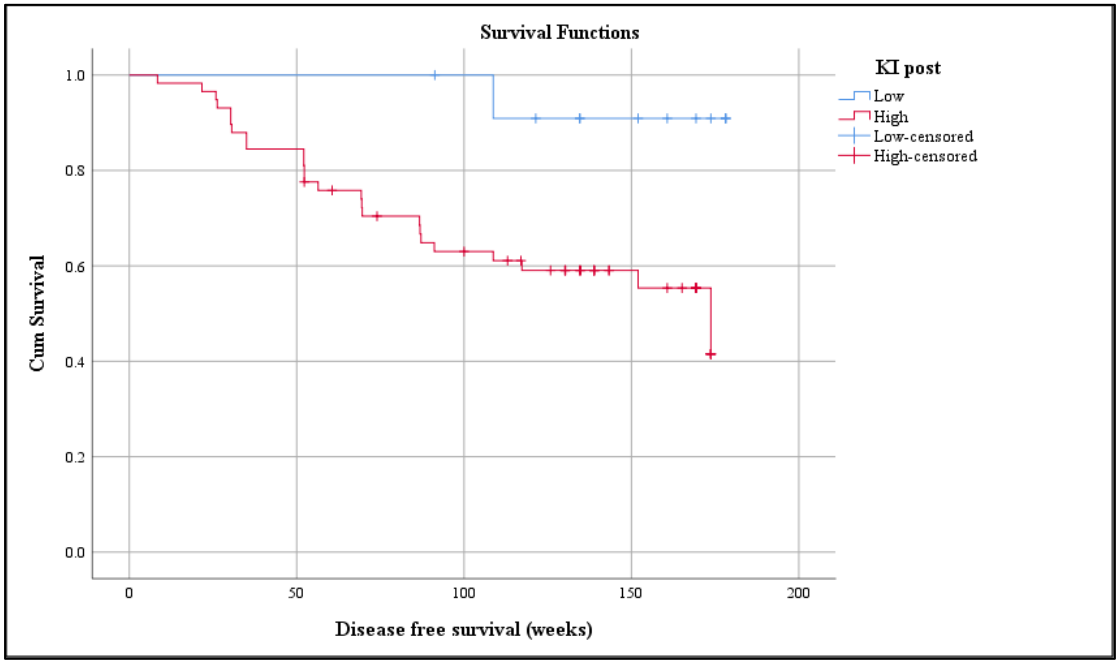


**Figure (1): shows low levels of ki67 post NAC associated with significant DFS.**

## Discussion

A pCR after NAC is strongly associated with favorable long-term outcomes[17]; however, patients who do not achieve pCR are a heterogeneous group with diverse prognoses, and unfortunately, until now, no definite biomarker has served as a prognostic discriminator. An even more difficult issue presents in patients with ER-positive BC, who tend to have low pCR rates and among whom it is highly challenging to distinguish patients with a good prognosis from those with a poor prognosis[18].

Such cases make it fundamental to acquire further tools that are urgently needed to assess potential outcomes.

This study was done to evaluate effect of NAC on patients with stage II and III breast cancer which was all IDC and the occurrence of pCR in different molecular subtypes and the change in HR and KI67.

In the current study, it is found that tumor cells that are hormone receptor negative are more sensitive to chemotherapy than hormone receptor-positive tumors. These results are in agreement with previous studies by Tan et al., [19], Caudle et al., [20], and Precht et al.,[21], which showed that hormone receptor-positive tumor cells, known as insensitive tumor cells, are left behind as part of the residual disease after NAC or have a higher proliferation index (Ki67).

This study also shows that Her2-positive tumor cells are more sensitive to chemotherapy and also more likely to be eliminated by chemotherapy. This result was in agreement with Quddus et al.,[22] and Wang et al.,[23] which showed that Her2-positive tumors are more sensitive to chemotherapy with an obvious effect on proliferation index rate and higher rate of achieving pCR.

Van De Ven et al.,[24] pointed out in a meta-analysis that HR may change in 8 to 33% of patients after NAC. Hirata et al., [25] reported that changes in ER and PR occurred in 23% of patients after NAC, but in our study only 10 patients had changed their hormonal status (14.3%).

In this study, we found that changes in Ki67 after NAC can be used to separate a subgroup of patients with better outcomes from the general BC population. It is quite common that, in the presence of a significant disease burden after NAC, clinicians

expect a high rate of recurrence even after completion of standard treatment (adjuvant chemotherapy and endocrine management, as well as radiotherapy), which leads to an increased use of additional non-evidence-based therapy[26]. A better selection of patients at high risk after NAC is important for the tailoring of new therapeutic strategies[27].

Since 1999, it has been reported that a decrease in the cell proliferation fraction has a predictive value with respect to the recurrence rate[28,29]. Ki67 has been used as a marker of such proliferation. Thus, routine assessment has not been recommended when patients receive primary chemotherapy because most data were derived from retrospective studies, and the cutoff points used were selected empirically or were arbitrarily established[30]. It has also been found that patients who experienced progression during NAC had a higher proliferation rate than those who responded to chemotherapy. It is also known that patients with high Ki67 expression at diagnosis have a higher risk of recurrence and death[31,32].

All of the above suggests that Ki67 may be used to define prognosis.
High Ki67 expression at baseline is significantly associated with improved pCR rates[33], primarily in the triple negative and HER2-positive BC subtypes[34]. The potential prognostic value of Ki67 after NAC is less well known 33. Our findings suggest that the reduction in Ki67 value after NAC compared with the baseline level is associated with a favorable prognosis, as previously demonstrated by other research groups[35].

Billgren et al., demonstrated that a decrease in ki 67 after the first course of chemotherapy significantly predicted a reduced risk of recurrence. Further studies added information on the role of Ki67 in predicting a pathological response [27]. In our study, we found that a decrease of at least one point of the percentage of Ki67-positive cells between the core biopsy sample and the surgical specimen after the completion of NAC was related to better DFS compared with no decrease in the percentage of Ki67-positive cells. These data are consistent with the study of Diaz-Botero et al., who previously reported that patients whose tumors had low Ki67 expression after NAC had better OS and DFS compared with those whose tumors maintained high Ki67 expression [37].

This study provides evidence that patients without a decrease in Ki67 expression after NAC had worse outcomes. Ingolf and Yoshioka reported that high Ki67 expression in post-treatment tumors was strongly correlated with poor DFS. Other studies corroborate that patients with high Ki67 values in the residual tumor after chemotherapy had worse outcomes in terms of recurrence and mortality[38,39].

In this regard, Ki67 might serve as a valuable prognostic marker for patients who do not achieve a pCR, but no clear evidence shows the optimal way to measure the changes in Ki67 after chemotherapy. We agree that Ki67 reflects the percentage of proliferating cells in the tumor[40] and that it is possible that the best way to measure this proliferation is as a continuous variable[41].

Our data suggest that the evaluation of Ki67 after neoadjuvant chemotherapy could act as a clinically available tool that might allow clinicians to stratify patients into those who could benefit from ‘‘complementary’’ treatment. Clinicians must therefore be made aware that there are data available that incline us to believe that patients with a poor prognosis can be timely identified, and therefore more therapeutic options be made available for them.

**In conclusion**, the treatment management and outcomes for the LABC patients highly variable; however, positive outcomes are achieved in the treatment. Response to CT is related to level of hormone receptor, proliferation index and Her-2 neu. Several studies have shown that prognostic parameters change with NAC. In our study there is significant decrease in Ki-67 proliferation index. The significant decrease in Ki-67 proliferation index suggests that the proliferation of malignant cells will be reduced by NAC. We believe that randomized clinical studies evaluating the

long-term outcomes of patients treated by NAC are required to determine the significance of the changes in ER, PR.

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