Factors Predicting Response in Breast Cancer Receiving Neoadjuvant Therapy and the Role of Ki67 Labeling Index

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ABSTRACT

Objective: To determine the predictive value of Ki67 on pathological complete response (pCR) of breast and axilla regions in breast cancer (BC) patients receiving neoadjuvant therapy (NAT).

Study Design: Descriptive study.

Place and Duration of the Study: Departments of Medical Oncology, Sirnak State Hospital, Aydin State Hospital, Manisa Celal Bayar University, and Dokuz Eylul University, from November 2010 to July 2022.

Methodology: PCR and various histopathological parameters were evaluated for BC patients receiving NAT. The Youden Index method was used to find the cut-off value for the Ki67 variable according to the receiver operating characteristic (ROC) curve. This value was obtained as 77.5. Breast and axillary responses were individually evaluated to assess response to NAT. Univariate and multivariate logistic regression analysis were used to predict both breast and axillary pCR.

Results: A total number of 280 females receiving NAT for BC were included in the study. Multivariate analysis for breast pCR to NAT showed that Ki67 index (>77.5 *vs* <77.5, p=0.047) was statistically significant marker. While Ki67 index was significant for breast pCR in both univariate and multivariate analyses, the same was not observed on axillary response (p=0.387).

Conclusion: High Ki67 level was significantly associated with breast pCR in BC patients receiving NAT, but a similar effect was not observed on axillary pCR. These findings suggest that breast and axilla tissues have a biological differences in treatment responses.

Key Words: *Axillary response, Breast cancer, Ki67 Labeling Index, Neoadjuvant therapy, pathological complete response.*

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INTRODUCTION

Breast cancer (BC) is the most common type of cancer among women worldwide and is the leading cause of cancer-related deaths.[1] Although the basis of treatment varies according to the stage, it includes surgery, radiotherapy and chemotherapy. However, neoadjuvant therapy (NAT) is increasingly associated with better clinical outcomes.[2] NAT plays a key role in BC treatment as it allows breast-preserving surgery, helps inoperable tumours become operable, defines patients with residual disease and high risk of relapse by allowing complementary adjuvant regiments particularly, in patients with triple-negative breast cancer (TNBC) or HER2 positive BC, and saving time for further genetic analyses.

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In addition to various parameters such as lymph node involvement, tumour size, Ki67 proliferation index, tumour grade, hormonal status, and lymphovascular and perinodal invasion that predict prognosis in BC, the disease also has several different histopathological and molecular sub-types.[2,3] Presence of such a high number of components is a proof that BC represents a quite heterogeneous phenotype. This means the patients intended for NAT should be very carefully selected, as there is always a risk of progression while the patient is on treatment. Patient and tumour characteristics, genetic tests, and inflammation parameters become helpful while considering individualised treatment options. However, there is still no established marker available for use.[1,3]

Ki-67 is a nuclear protein produced out of G0 phase during the cell cycle and it is a marker of proliferating cells.[4-7] It was initially investigated in Hodgkin lymphoma by the end of the 20th century, whilst it became more popular after being shown to be essential for breast cancer. After its relation with both luminal differences and treatment response was demonstrated, studies mostly concentrated on these aspects.[5] However, the most obvious handicap for the use of Ki67 proliferation index is that the established threshold value varies significantly

between the studies. On the other hand, routine use is possible because Ki67 values are mostly reported in breast cancer histopathology reports.[8] The most important detail to focus on here is that the interpretation of these values can be complicated as NAT can have an impact on postoperative assessments. The objective of this study was to determine the predictive value of Ki67 on pCR of breast and axilla regions in BC patients receiving NAT.

METHODOLOGY

The data of BC patients registered in the Departments of Medical Oncology, Sirnak State Hospital, Aydin State Hospital, Manisa Celal Bayar University and Dokuz Eylul University, from November 2010 to July 2022 and receiving NAT were retrospectively analysed. Patients older than 18 years of age, who were operated on after NAT, and whose pathological evaluation was sufficient in terms of immune histochemical parameters were included in this study. Patients with a diagnosis of cancer other than breast, stage 4 disease, patients who could not complete NAT, and male gender were excluded from the study.

The study was designed as a multicentre retrospective cohort. Age, body mass index (BMI), comorbidities (diabetes mellitus, coronary artery disease, and chronic lung disease), menopausal status, smoking, primary tumour lateralization, clinical tumoural and nodal stage, CA 15-3, histological grade, pathological subtypes (invasive ductal carcinoma, invasive lobular carcinoma, and others), molecular subgroup (luminal A, luminal B (HER2 negative), HER2 positive, triple negative), lymphovascular and perinodal invasion, estrogen receptor and progesterone receptor status, Her2 positivity (c-erb B2 +3 by immunohistochemistry or positivity by fluorescence *in situ* hybridization), Ki67 level were noted. Antitumour drugs (anthracycline, cyclophosphamide, taxane, carboplatin, trastuzumab, pertuzumab) taken for NAT by molecular subgroups were also reported.

Histopathological grouping was done in three groups as ductal type, lobular type, and others. Those with hormone positivity ER (estrogen receptor) or PR (progesterone receptor) above 1% were considered positive. Cases with a c-erb B2 score of +3 after immunohistochemical (IHC) analysis or positive with Fluorescent *in situ* Hybridization (FISH) analysis were considered Her2 positive. The Ki-67 cut-off value for luminal separations was taken as 14. Therefore, luminal distinctions were made according to the cut-off value.[6] Tumour pathological staging was performed according to the AJCC TNM-8 classification. Molecular subtype groups were labelled as Luminal A [Hormone receptor-positive (ER and/or PR positive), Ki67<14 and Her2 negative], Luminal B [Hormone receptor-positive (ER and/or PR positive), Ki67>14 and HER2 negative], HER2 positive (HER2 positive regardless of hormonal status), and triple-negative (all ER, PR, and HER2 receptors negative).[6]

The patients received 4 cycles of docetaxel (every 14-21 days) or paclitaxel (12 cycles if weekly or 4 cycles every 21 days) after the combination of four cycles of cyclophosphamide and anthracycline (every 14-21 days). Because there are different applications in different centres some triple-negative patients received carboplatin therapy in combination with a taxane (docetaxel or paclitaxel), according to clinician preference. Patients with human epidermal growth factor receptor 2 positive (HER2+) were given trastuzumab (12 cycles in a week or 4 cycles in 21 days) in the neoadjuvant period, and pertuzumab (4 cycles in 21 days) was given to some patients, however, since repayment conditions were not possible in all patients in the author's country.

Statistical analysis was performed with the SPSS 18 (Chicago: SPSS Inc) package program. The Youden Index method was used to find the cut-off value for the Ki67 variable according to the ROC curve, and this value was obtained as 77.5. As a result of the examination of the pathology preparations in patients who were operated on after NAT, two different possible responses were categorised. If no viable tumour tissue was present, pathological complete response (pCR) was classified and no pathological complete response for all other possibilities, including microscopic residual tumour. Breast response (BR) and axilla response (AR) are discussed separately. Tumour pathological staging was performed according to the AJCC TNM classification.[2]

Univariate and multivariate logistic regression analysis was used to predict both breast and axillary pCR. Logistic regression results were reported using odds ratio (OR) with a 95% confidence interval (CI). Continuous variables were expressed as mean and standard deviation and the categorical variables were expressed as counts and percentages. Independent sample test (for continuous variables) or chi-square tests (for categorical variables) was used. For all statistical results, a p-value of <0.05 was considered statistically significant.

RESULTS

In total, 280 women receiving NAT were included in the study. The mean age of the patients was 50.91 ± 11.73 years, ranging from 26 - 86 years, and the majority was older than 40 years (83.2 %, n=233, Table I). Since only response to NAT is evaluated, the median duration of follow-up was 6.40 ± 0.218 (95% CI=5.97-6.83) months. When the patients were classified based on the Ki67 cut-off value estimated by ROC analysis, 24 patients had Ki67 values higher than the cut-off (>77.5) while the values were lower among the remaining 256 patients (<75.5). When the patients were compared based on Ki67 cut-off values, significant differences were noted between the groups (<77.5 *vs.* >77.5) in ER status, PR status, molecular subgroup, multifocality, and pCR to NAT (breast) ($p<0.001$, $p=0.001$, $p<0.001$, $p=0.026$, and $p=0.047$, respectively). Logistic regression analysis performed for breast pCR to NAT showed statistically significant results for age ($p=0.037$), nodal stage ($p=0.014$), ER ($p=0.001$), and PR ($p=0.001$) status, molecular subgroup (HER2 positive and triple negative *vs.* luminal A, $p=0.021$ and $p=0.015$), lymphovascular invasion ($p<0.001$), perineural invasion ($p=0.011$), multifocality ($p=0.045$), trastuzumab therapy ($p=0.007$), and Ki67 index ($p=0.032$).

Table I: Comparison of clinical characteristics and treatment conditions according to the Kİ67 cut-off value.

		<77.5 (n-%)	>77.5 (n-%)	Total (n-%)	p-value
Age category	<40 years old	42 (16.4)	5 (20.8)	47 (16.8)	0.570
	≥40 years old	214 (83.6)	19 (79.2)	233 (83.2)	
DM	No	222 (86.7)	21 (87.5)	243 (86.8)	1.000
	Yes	34 (13.3)	3 (12.5)	37 (13.2)	
CAD	No	238 (93.0)	23 (95.8)	261 (93.2)	1.000
	Yes	18 (7.0)	1 (4.2)	19 (6.8)	
Chronic lung disease	No	230 (89.8)	22 (91.7)	252 (90.0)	1.000
	Yes	26 (10.2)	2 (8.3)	28 (10.0)	
Menopausal status	Postmenopausal	130 (50.8)	10 (41.7)	140 (50.0)	0.522
	Premenopausal	126 (49.2)	14 (58.3)	140 (50.0)	
Tumoral stage	1	3 (1.2)	0 (0)	3 (1.1)	0,725
	2	112 (43.8)	9 (37.5)	121 (43.2)	
	3	138 (53.9)	15 (62.5)	153 (54.6)	
	4	3 (1.2)	0 (0)	3 (1.1)	
Nodal stage	0	18 (7.0)	2 (8.3)	20 (7.1)	0.649
	1	185 (72.3)	17 (70.8)	202 (72.1)	
	2	44 (17.2)	4 (16.7)	48 (17.1)	
	3	9 (3.5)	1 (4.2)	10 (3.6)	
Tumor lateralization	Right	129 (50.4)	9 (37.5)	138 (49.3)	0.481
	Left	118 (46.1)	14 (58.3)	132 (47.1)	
	Bilateral	9 (3.5)	1 (4.2)	10 (3.6)	
ER status	Positive	186 (72.7)	3 (12.5)	189 (67.5)	0.000
	Negative	70 (27.3)	21 (87.5)	91 (32.5)	
PR status	Positive	126 (49.2)	3 (12.5)	129 (46.1)	0.001
	Negative	130 (50.8)	21 (87.5)	151 (53.9)	
C-ErbB2 (+3) or FISH positivity	Yes	99 (38.7)	7 (29.2)	106 (37.9)	0.485
	No	157 (61.3)	17 (70.8)	174 (62.1)	
Histological grade	1	55 (21.5)	9 (37.5)	64 (22.9)	0.089
	2	56 (21.9)	1 (4.2)	57 (20.4)	
	3	46 (18.0)	5 (20.8)	51 (18.2)	
	Unknown	99 (38.7)	9 (37.5)	108 (38.6)	
Pathological subtype	Invasive ductal carcinoma	208 (81.3)	20 (83.3)	228 (81.4)	0.090
	Invasive lobular carcinoma	27 (10.5)	0 (0)	27 (9.6)	
	Other pathological subtypes	21 (8.2)	4 (16.7)	25 (8.9)	
Molecular Subgroup	luminal A	60 (23.4)	2 (8.3)	62 (22.1)	0.000
	luminal B	75 (29.3)	2 (8.3)	77 (27.5)	
	HER2 positive	85 (33.2)	6 (25.0)	91 (32.5)	
	Triple negative	36 (14.1)	14 (58.3)	50 (17.9)	
Lymphovascular invasion	No	97 (37.9)	7 (29.2)	104 (37.1)	0.532
	Yes	159 (62.1)	17 (70.8)	176 (62.9)	
Perineural invasion	No	208 (81.3)	21 (87.5)	229 (81.8)	0.586
	Yes	48 (18.8)	3 (12.5)	51 (18.2)	
Multifocality	No	148 (57.8)	20 (83.3)	168 (60.0)	0.026
	Yes	108 (42.2)	4 (16.7)	112 (40.0)	
Anthracycline therapy	No	16 (6.3)	2 (8.3)	18 (6.4)	0.659
	Yes	240 (93.8)	22 (91.7)	262 (93.6)	
Cyclophosphamide therapy	No	16 (6.3)	1 (4.2)	17 (6.1)	1.000
	Yes	240 (93.8)	23 (95.8)	263 (93.9)	
Taxane therapy	No	19 (7.4)	1 (4.2)	20 (7.1)	1.000
	Yes	237 (92.6)	23 (95.8)	260 (92.9)	
Carboplatin therapy	No	245 (95.7)	22 (91.7)	267 (95.4)	0.308
	Yes	11 (4.3)	2 (8.3)	13 (4.6)	
Trastuzumab therapy	No	171 (66.8)	19 (79.2)	190 (67.9)	0.311
	Yes	85 (33.2)	5 (20.8)	90 (32.1)	
Pertuzumab therapy	No	228 (89.1)	20 (83.3)	248 (88.6)	0.497
	Yes	28 (10.9)	4 (16.7)	32 (11.4)	
Complete response to neoadjuvant therapy (Breast)	No	165 (64.5)	10 (41.7)	175 (62.5)	0.047
	Yes	91 (35.5)	14 (58.3)	105 (37.5)	
Complete response to neoadjuvant therapy (Axilla)	No	141 (55.1)	11 (45.8)	152 (54.3)	0.512
	Yes	115 (44.9)	13 (54.2)	128 (45.7)	

The relationship between clinicopathological results and Ki67 index was evaluated with the chi-square test. BR: Breast response, AR: Axilla response, OR: Odds ratio, Min: Minimum, Max: Maximum, BMI: Body mass index, DM: Diabetes mellitus, CAD: Coronary artery disease, ER: Estrogen receptor, PR: Progesterone receptor, FISH: Fluorescence in situ hybridisation, ULN: Upper limit of normal.

Table II: Univariate logistic regression analysis results of BR and AR in breast cancer patients receiving neoadjuvant therapy.

	Univariate BR OR (95% CI) (Min-max)	p-value	Univariate AR OR (95% CI) (Min-max)	p-value
Age	0.994 (0.974-1.015)	0.591	0.997 (0.977-1.017)	0.766
Age category				
<40 years old vs ≥40 years old	0.511 (0.271-0.961)	0.037	0.697 (0.372-1.307)	0.261
DM	0.889 (0.431-1.832)	0.75	1.011 (0.505-2.024)	0.976
CAD	1.23 (0.478-3.163)	0.668	0.855 (0.333-2.194)	0.744
Chronic lung disease	0.77 (0.335-1.77)	0.538	0.879 (0.4-1.934)	0.749
Postmenopausal vs premenopausal	1.031 (0.635-1.673)	0.902	0.891 (0.557-1.427)	0.631
Tumoral stage1		0.535 0.72		0.343
Tumoral stage 2	0.9 (0.506-1.602)	0.295	0.874 (0.496-1.539)	0.64
Tumoral stage 3	0.612 (0.244-1.533)	0.243	0.765-0.322-1.815) 0.357	0.543
Tumoral stage 4	0.51 (0.165-1.579)		(0.116-1.102)	0.073
Nodal stage 0		0.09		0.006
Nodal stage 1	0.298 (0.114-0.781)	0.014	0.088 (0.02-0.387)	0.001
Nodal stage 2	0.269 (0.09-0.807)	0.019	0.067 (0.014-0.322)	0.001
Nodal stage 3	0.359 (0.075-1.714)	0.199	0.048 (0.007-0.349)	0.003
Tumor lateralization (Right)		0.348		0.058
Left	1.257 (0.769-2.056)	0.361 0.33	1.704 (1.051-2.761)	0.03
Bilateral	0.454 (0.093-2.223)		0.647 (0.16-2.609)	0.54
ER negative vs positive	2.413 (1.442-4.035)	0.001	2.6 (1.555-4.349)	0
PR negative vs positive	2.321 (1.404-3.838)	0.001	2.422 (1.491-3.935)	0
Ki67 >77.5 vs <77.5	2.538 (1.084-5.944)	0.032	1.449 (0.626-3.356)	0.387
C-ErbB2 (+3) or FISH positive	0.628 (0.382-1.031)	0.066	0.434 (0.265-0.71)	0.001
CA 15-3 >ULN	0.997 (0.987-1.006)	0.483	0.993 (0.982-1.005)	0.261
Histological grade 1		0.657		0.994
Histological grade 2	0.72 (0.34-1.526)	0.391	1.086 (0.531-2.222)	0.821
Histological grade 3	1.183 (0.56-2.5)	0.659	0.991 (0.473-2.076)	0.982
Histological grade unknown	0.918 (0.486-1.734)	0.791	1.002 (0.539-1.865)	0.994
Invasive ductal carcinoma		0.655		0.394
Invasive lobular carcinoma	0.67 (0.281-1.596)	0.366	0.701 (0.308-1.598)	0.398
Other pathological subtypes	0.895 (0.379-2.113)	0.8	1.517 (0.661-3.486)	0.326
Molecular Subgroup (luminal A)		0.007		0.001
Luminal B	0.993 (0.469-2.102)	0.985	0.995 (0.491-2.018)	0.99
HER2 positive	2.269 (1.134-4.54)	0.021	2.849 (1.455-5.58)	0.002
Triple negative	2.647 (1.206-5.812)	0.015	2.292 (1.066-4.928)	0.034
Lymphovascular invasion	0.404 (0.244-0.667)	0	0.313 (0.189-0.519)	0
Perineural invasion	0.395 (0.193-0.809)	0.011	0.339 (0.172-0.67)	0.002
Multifocality	0.596 (0.359-0.988)	0.045	0.731 (0.451-1.185)	0.203
Operation	0.955 (0.587-1.553)	0.852	0.689 (0.429-1.107)	0.123
Anthracycline therapy	1.605 (0.555-4.638)	0.382	1.349 (0.507-3.587)	0.549
Cyclophosphamide therapy	1.107 (0.397-3.086)	0.846	2.899 (0.921-9.125)	0.069
Taxane therapy	1.435 (0.534-3.856)	0.474	2.063 (0.769-5.534)	0.15
Carboplatin therapy	1.044 (0.332-3.278)	0.942	1.019 (0.333-3.112)	0.974
Trastuzumab therapy	2.023 (1.211-3.38)	0.007	2.699 (1.609-4.528)	0

All variables were insignificant (p-value >0.05) in the logistic regression univariate analysis for pathological complete response. BR: Breast response, AR: Axilla response, OR: Odds ratio, Min: Minimum, Max: Maximum, BMI: Body mass index, DM: Diabetes mellitus, CAD: Coronary artery disease, ER: Estrogen receptor, PR: Progesterone receptor, FISH: Fluorescence in situ hybridisation, ULN: Upper limit of normal.

Table III: Multivariate logistic regression analysis results of BR and AR in breast cancer patients receiving neoadjuvant therapy.

	Multivariate BR OR (95% CI) (Min-max)	p-value	Multivariate AR OR (95% CI) (Min-max)	p-value
Age category				
<40 years old vs ≥40 years old	0.504 (0.256-0.991)	0.047		
Nodal stage 0		0.06		0
Nodal stage 1	0.255 (0.092-0.712)	0.009	0.041 (0.008-0.204)	0
Nodal stage 2	0.215 (0.066-0.705)	0.011	0.022 (0.004-0.126)	0
Nodal stage 3	0.293 (0.055-1.573)	0.152	0.013 (0.001-0.121)	0
ER negative vs positive	2.126 (1.23-3.674)	0.007		
PR negative vs positive			2.137 (1.219-3.747)	0.008
C-ErbB2 (+3) or FISH positive			0.394 (0.222-0.698)	0.001
Ki67 >77.5 vs <77.5	2.584 (1.015-6.582)	0.047		
Lymphovascular invasion	0.409 (0.237-0.706)	0.001	0.274 (0.151-0.498)	0
Perineural invasion			0.452 (0.203-1.007)	0.052
Multifocality	0.621 (0.359-1.074)	0.088		
Trastuzumab therapy	1.748 (1.006-3.038)	0.048		
Tumor lateralization (Right)		0.008		
Left	2.437 (1.362-4.359)	0.003		
Bilateral	0.74 (0.153-3.58)	0.709		

All variables were insignificant (p-value >0.05) in the logistic regression multivariate analysis for pathological complete response. BR: Breast response, AR: Axilla response, OR: Odds ratio, Min: Minimum, Max: Maximum, ER: Estrogen receptor, PR: Progesterone receptor, FISH: Fluorescence in situ hybridisation.

When the same parameters were evaluated by multivariate analysis, statistically significant differences were obtained for age, nodal stage, ER positivity, lymphovascular invasion, trastuzumab therapy, tumour lateralization and Ki67 index. When the response to NAT was classified into two groups as breast and axilla response, logistic regression analysis performed for axilla response demonstrated different results than breast response (Tables II and III). However, Ki67 index was not found to be significant in neither univariate nor multivariate analysis as a marker of axilla pCR.

DISCUSSION

The present study investigated the predictive value of Ki67 index for breast and axilla pCR in BC patients receiving NAT. While both univariate and multivariate analysis showed that Ki67 index was a significant marker of breast pCR (p=0.032, and p=0.047, respectively), the same result was not shown for the axilla region (p=0.387). These findings suggest that breast and araxilla tissues have biology that leads to different treatment responses.

In a quite recent study investigating the factors affecting pCR in 183 BC patients receiving NAT, tumours with aggressive characteristics such as high Ki67 (<0.001), high grade (<0.001), and Her2 positivity (p=0.045) were associated with better response rates.[9] In that study performed by Müller *et al*., pCR was defined as the absence of residual tumour in the breast and axilla, which is different from the author's definition. For instance, multivariate analysis in this study showed that age <40 years old *vs.* ≥40 years old (p=0.047), ER-negative *vs.* positive (p=0.007), Ki67 >77.5 *vs.* <77.5 (p=0.047), trastuzumab therapy (p=0.048) and tumour lateralisation (left *vs.* right, p=0.003), were only significantly associated with breast pCR, while nodal stage 3 (p<0.001), PR negativity positive (p=0.008) and C-ErbB2 (+3) or FISH positivity (p=0.001) were only significantly associated with axilla pCR. In addition, pCR rate was calculated as 47% in the study of Muller *et al*., it was higher in this study with 37.5% breast and 45.7% axillary pCR rates. In light of this information, classifying pCR as breast and axilla may help us to make better prognosis estimates.

Another study performed by Shi *et al.* in 2021 included 184 patients with triple-negative and positive 184 BC and investigated predictive factors to avoid axillary dissection in patients with complete axillary response. That study is similar to the present, as it was also designed to classify complete response to breast and axillary response. Multivariate analysis investigating factors associated with complete axillary response after NAT showed that clinical lymph node staging (p<0.001), clinically LN-negative disease after NAT (p<0.001) and radiological full-response in breast (p<0.001) were significantly associated with axillary complete response. In line with the present findings, Ki67 level (p=0.255) was not a predictor of axillary complete response in that study.[10] It should also be emphasised that the cut-off Ki67 level in that study was also different.

Another study including 353 BC patients receiving NAT evaluated several parameters for their potential effects on pCR. While the univariate analysis showed statistically significant effects of several parameters such as tumour grade (p<0.001), nuclear grade (p<0.001), mitotic index (p<0.001), Ki67 (p<0.001), estrogen and progesterone receptor (p<0.001), and triple-negative status (p=0.003), multivariate analysis indicated only tumour grade (p=0.017) and estrogen receptor status (p=0.0475) were independent variables.[11] We have demonstrated in the present study that Ki67 levels were significantly associated with breast pCR in both univariate and multivariate analyses (p=0.032 and p=0.047, respectively).

In another recent study performed by Jain *et al*., Ki-67 index was meticulously investigated as a marker of NAT response in BC patients. ROC analysis demonstrated Ki67 cut-off value of 35%, and the relation with both clinical complete response (cCR) and pCR was further analysed. The study involved 134 patients in total and pCR was defined as the absence of pathological proof of residual invasive carcinoma in the breast or axillary lymph nodes. Both univariate and multivariate analyses in that study demonstrated that Ki67 was an independent and strong predictive index for cCR (p=0.002, p=0.048, respectively) as well as pCR (p<0.001 and p=0.011, respectively).[12] That study had low cCR (26.1%) and pCR (23.9%) rates, although the literature overall shows varying response rates.[13-18] The most probable cause of this variation is the differences between patient-specific treatment protocols as well as molecular subtypes of the disease.

In another recent study including BC patients receiving NAT, 359 development cohort and 351 validation cohort were involved to design a nomogram to predict pCR and the results showed that hormone receptor negativity (p=0.006), high Ki-67 index (p<0.001), and post-NAC MRI variables, including small tumour size (p=0.03), low lesion-to-background parenchymal signal enhancement ratio (p=0.004), and absence of enhancement in the tumour bed (p=0.009) were independently associated with pCR. The nomogram which was prepared considering all these predictive factors showed good discrimination and calibration abilities in predicting pCR. The fact that Ki-67 index was used in the nomogram created in this quite recent study shows that it is still valuable in the assessment of NAT response.[19]

A study performed by Mukai *et al.* in 2020 had a remarkable phase 2, prospective, double-arm study design. In the HER 2 positive-subgroup, one arm was given standard 12 cycles of paclitaxel and trastuzumab treatment irrespective of control Ki67 values, while in the other arm, the treatment was stopped and replaced with epirubicin and cyclophosphamide in patients whose Ki67 levels did not decline based on

repetitive measurements made after initiation of treatment. This analysis involving 237 patients showed an almost linear correlation between Ki-67 decline rates and pCR rates. The pCR rate in those without early Ki-67 response in Ki-67 group was found to be lower than the rates in the control group (p=0.025).[20]

The significant limitations of this study were that it was designed retrospectively, and the Ki67 index does not have the ideal cut-off value that prevents routine use. Further studies involving larger patient groups with analyses in the histopathological subtypes of BC will reinforce the present findings.

CONCLUSION

High Ki67 level was a significant predictor of breast pCR in BC patients receiving NAT, in both the univariate and multivariate analyses but a similar effect was not observed on axillary response. These findings suggest that breast and axilla tissues have biology that causes differences in treatment responses.

ETHICAL APPROVAL:
The Ethics Committee approved this study of the University, Faculty of Medicine with the decision dated 09/02/2022 and No: E-61804347-100-247244.

PATIENTS' CONSENT:
Patients' consent for inclusion in this study and using their data in publishing the study was obtained prior to commencing this study.

COMPETING INTEREST:
The authors declared no competing interest.

AUTHORS' CONTRIBUTION:
FE: Literature search, study design, and facilitating conduction of study.
MU: Study design, statistical assistance, and conduction of the study.
BD: Study design, drafting, revisions, and editing.
APE: Study conduction and design.
ITU: Study design, conduction, statistical assistance, and manuscript editing.
All the authors have approved the final version of the manuscript to be published.

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