Original Article

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Role of Ki-67 in Carcinoma Breast as Predictive Marker of Pathological Response to Neoadjuvant Chemotherapy: A Cross-sectional Study

Pathology Section

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ABSTRACT

Introduction: Breast carcinoma is the most common invasive cancer in female gender. Neoadjuvant Chemotherapy (NAC) helps to achieve resectability. The pathological response to NAC is classified as a pathological Complete Response (pCR), pathological Partial Response (pPR) and pathological No Response (pNR).

Aim: To evaluate the role of Ki-67 as a predictive marker of pathological response and to find the optimum percentage of Ki-67 positivity that can be associated with pCR.

Materials and Methods: The present cross-sectional study was conducted in the Department of Pathology, Government Medical College Thrissur, Kerala, India, between March 2021 and January 2022, which involved 50 breast carcinoma patients. Fifty patients who had undergone mastectomy post-NAC were selected. Ki-67 immunohistochemical staining was done on the initial trucut biopsy sample of the patients. Post-NAC mastectomy specimens were evaluated for tumour clearance. Association of Ki-67 score with pathological response in the mastectomy specimen was studied. Percentage cut-off for Ki-67 in initial trucut biopsy of breast, that could effectively predict pCR in the post-NAC mastectomy specimens was derived by Receiver Operating Characteristic (ROC) curve analysis.

Results: Total 50 cases of breast cancer were studied with mean±Standard Deviation (SD) age of 53.3±10.3 years. Eight (16%) out of 50 patients had achieved pCR while, 18 out of 50 patients (36%) showed pPR and 24 out 50 patients (48%) had pNR. Significant association between Ki-67 score and pathological response (p-value=0.03) was found. Optimal percentage cut-off for Ki-67 that could predict pCR was found to be 40% (p-value=0.023).

Conclusion: The Ki-67 can be used as an independent predictive marker of pathological response in patients undergoing NAC. Ki-67 value of more than 40% shows strong association with pCR.

Keywords: Immunohistochemistry, Mastectomy, Treatment response, Trucut biopsy

INTRODUCTION

Breast carcinoma is the most common invasive cancer in females, accounting up to 11.7% of all malignancies [1]. NAC for locally advanced breast cancer and inflammatory carcinoma aids in downstaging and to achieve resectability [2]. The histological response to NAC is defined and classified as per National Surgical Adjuvant Breast and Bowel Project B-18 (NSABP-B 18), Food and Drug Administration (FDA) and American Joint Committee on Cancer (AJCC) as pCR, when there is no evidence of residual invasive tumour in the breast or axillary lymph nodes; as pPR, when there is presence of viable tumour cells in the presence of associated treatment changes and as pNR, when there is viable tumour cell in the absence of any therapy related changes [3-5]. However, the pathological response to chemotherapy is variable from patient to patient and depends on factors such as hormone receptor status and histomorphological parameters [6-9].

Molecular classification subcategorises the breast carcinoma into the following groups: luminal A and B, Human Epidermal growth factor Receptor 2 (HER2) enriched and basal, based on the expression of Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2, which are routinely evaluated prior to initiation of chemotherapy to predict the response to treatment [10]. The percentage of Ki-67 positive tumour cells determined by Immunohistochemistry (IHC) is often used to stratify patients into good and poor prognostic groups with suggested threshold of 14-15% to discriminate between cases, which likely correlate with more aggressive luminal B cell type with Ki-67 >14 or 15% and luminal A with Ki-67 <14 or 15% [11]. But ambiguity remains regarding scoring, definition of low versus high expression and appropriate cut-off point for positivity that could predict the complete pathological response. There is also a paucity of data on the effects of preanalytic variables such as length of fixation, ischaemic time or antigen retrieval which can interfere with Ki-67 assessment.

If Ki-67 could effectively predict the complete pathological response in breast carcinoma, tumour reduction by NAC may be beneficial in such patients. There are limited studies on Indian populations to justify the role of Ki-67 to predict pathological response which highlights the need for such a study [12]. Hence, the present study was aimed to evaluate the role of Ki-67 as a predictive marker of pathological response to NAC and to find the optimal percentage cut-off for Ki-67 in initial trucut biopsy of breast, that could effectively predict pathological response in the post-NAC mastectomy specimens.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Pathology, Government Medical College, Thrissur, Kerala, India, between March 2021 and January 2022. Study was conducted 50 breast carcinoma patients after getting approval from the Institutional Ethics Committee (Ref No: IEC/GMCTSR/038/2021).

Sample size calculation: Sample size was calculated with the formula of sample size using the formula=$(Z\alpha)^2 \times S(1-S)/d2$ p; $Z\alpha$=1.96 (constant). As per study conducted by Tan QX et al., S=86%, p=11.6%, d=0.05 [13]. Thus, the calculated minimal sample size for the study was 16 and sample size of 50 were included.

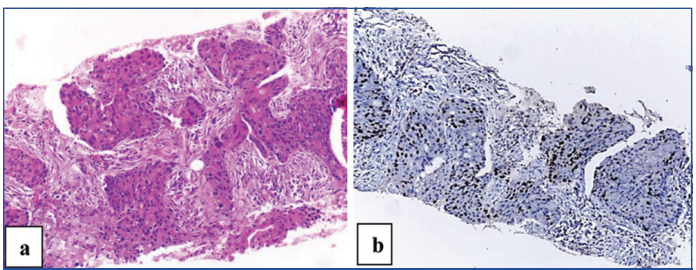
Inclusion criteria: Patients with a prior histological diagnosis of carcinoma breast on trucut biopsy and had received atleast four cycles of NAC prior to mastectomy were included in the study.

Exclusion criteria: Patients who had received incomplete preoperative NAC, non epithelial malignancy of the breast, cases with known malignancies of other organs and recurrent breast carcinomas were excluded from the study.

Study Procedure

Patients were selected based on the inclusion criteria, after obtaining consent, when their post-NAC wide excision or mastectomy samples were received in the study department for assessment of pathological response. The initial trucut biopsy from breast lump (pre-NAC) was reassessed for histological diagnosis. A 4 µm thick section was taken for Haematoxylin and Eosin (H&E) staining and IHC staining from formalin fixed paraffin embedded tissue. IHC analysis for Ki-67 performed on breast core biopsy, using an immunoenzymatically soluble complex method which includes the following steps: antigen retrieval using Ethylenediaminetetraacetic Acid (EDTA) antigen retrieval buffer in Multi Epitope Retrieval System (MERS), blocking of endogenous peroxidase using hydrogen peroxide followed by treatment with primary antibody, for Ki-67, mouse monoclonal MIB1 antibody, polyexcel target binder and polyexcel horseradish peroxidase and 3,3′-Diaminobenzidine (DAB) chromogen. Washing using distilled water and tris-buffered saline wash buffer was carried out after each step. Counterstaining was done with haematoxylin and the slides were dehydrated, cleared and mounted.

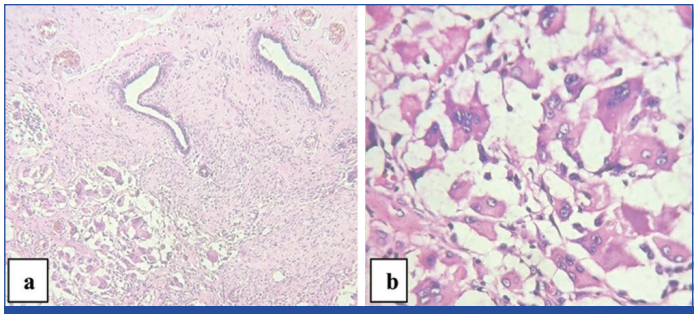
The Ki-67 staining was evaluated as nuclear staining using regular light microscope at the magnification of 40X. Ki-67 was scored as the percentage of positive tumour cell nuclei by counting a range of 1000 cells (depending on the cellularity of the specimen), 500 cells in two foci each, including also hot spot areas [Table/Fig-1a,b]. Hot spots were defined as areas in which Ki-67 staining is particularly prevalent, in an otherwise homogeneously stained area. The post-NAC mastectomy specimen was fixed in 10% neutral buffered formalin and routinely processed, with sections taken from tumour bed, deep resected margin, nipple, areola, overlying skin and any suspicious areas. Lymph nodes less than 0.5 cm were fully submitted, while those more than 0.5 cm was submitted in half. H&E section was studied for residual tumour. Pathological response was classified as per NASBP-B 18, Food and Drug Administration (FDA) and American Joint Committee on Cancer (AJCC) [3-5]; pCR- no recognisable invasive tumour cells present [Table/Fig-2a,b], pPR- presence of viable tumour cells in the presence of associated treatment changes such as nuclear changes (vacuolation, pyknosis and multinucleation), cytoplasmic changes (vacuolation) and stromal changes (fibrosis, collagenisation and vascular hyalinisation) [Table/Fig-3] and pNR- tumours not exhibiting treatment related changes. Incomplete response includes pNR and pPR. Thus, the pathological response in the mastectomy specimen (post-NAC) was evaluated for association with Ki-67 score obtained in the initial trucut biopsy (pre-NAC) of the patient.



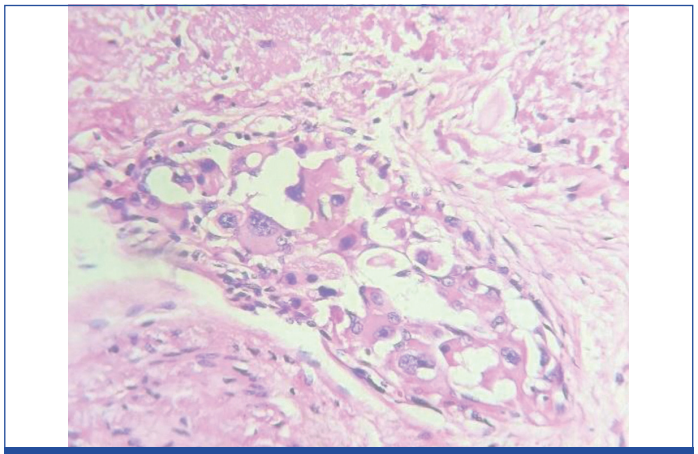
[Table/Fig-1]: a) Invasive carcinoma-NST (H&E, 20X); b) Ki-67 score: 40% (IHC, 20X).

STATISTICAL ANALYSIS

Collected data was analysed by International Business Machines-Statistical Package for the Social Sciences (IBM-SSPS) software version 20.0. Results on continuous measurements were presented as mean±SD, while on categorical measurements were expressed in figures and percentages. Significance was assessed at 5% level of significance. Kruskal-Wallis test was conducted to find out any statistically significant difference in the distribution of Ki-67 among the three pathological response groups. Pathological response was recategorised into two groups as complete pCR, and non complete responders (non pCR), which includes partial pathological response and no pathological response groups, and Fisher's-exact was done to find out any statistically significant difference between these two groups. Sensitivity, specificity, positive likelihood, and negative likelihood ratios were assessed. The p-value <0.05 was considered statistically significant. ROC curve analysis was done calculate the optimal percentage cut-off for Ki-67, after dividing the response groups as pCR and non pCR and Ki-67 values of each case belonging to the two groups were plotted.



[Table/Fig-2]: a) Section from post-NAC mastectomy specimen with no residual neoplasm (H&E, 20X); b) Extensive chronic inflammation with foreign body giant cell reaction (H&E, 40X).



[Table/Fig-3]: Microscopic foci of residual neoplasm showing cytoplasmic vacuolation bizarre nuclei consistent with partial pathological response (H&E, 40X).

RESULTS

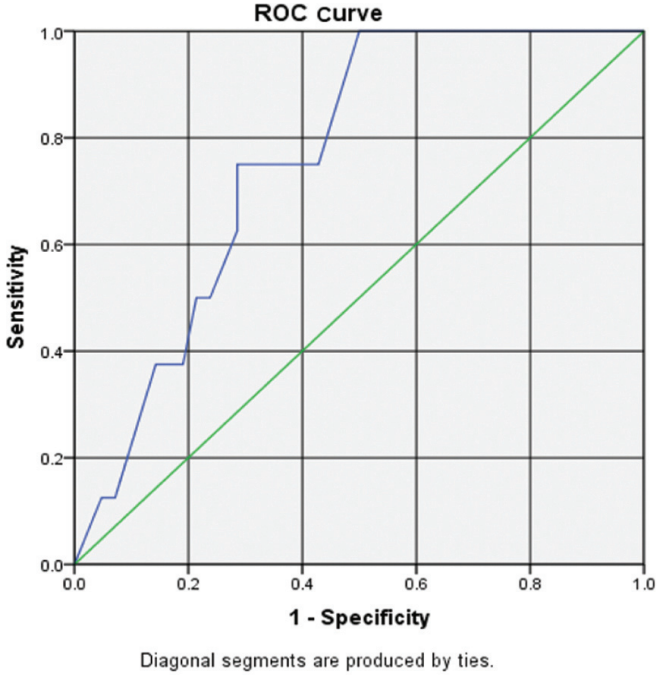
The mean±SD age of the study participants was 53.3±10.3 years (range: 32-78 years). Eight (16%) out of 50 patients had achieved pCR, while 18 (36%) out of 50 patients showed pPR and 24 (48%) out 50 patients had pNR. Kruskal-Wallis test showed that there was a statistically significant difference in the distribution of Ki-67 among pathological responses (p-value <0.05) [Table/Fig-4]. Posthoc test also confirmed that there was a statistically significant difference between pCR and non pCR (p-value <0.05).

Parameter	pCR (n=8)	pPR (n=18)	pNR (n=24)	Total (n=50)	p-value
Ki-67 (Mean±SD)	56.25±25.04	35.67±25.75	29.5±28.15	36.0±27.91	**0.03**

[Table/Fig-4]: Comparison of Ki-67 (%) distribution between pathological response groups.
The p-value in bold font indicates statistcially significant value

By ROC curve, Area Under the Curve (AUC) was found to be significant (AUC: 0.760, p-value=0.021) and Ki-67 equal to or greater than 40% was calculated as the most accurate cut-off point, (sensitivity 75%, specificity 71.4%, positive likelihood ratio=2.62,

negative likelihood ratio=0.35) at which or above, was considered to have strong association with pCR [Table/Fig-5]. Based on the ROC curve, Ki-67 is categorised into two groups as ≥40% and <40% and Fisher's-exact test showed that there was significant difference between pCR and non pCR groups [Table/Fig-6]. Logistic regression was also done to check the predictive contribution of Ki-67 and confirmed that there is a strong association of Ki-67 with pathological response (OR=7.5, 95% CI: 1.323-42.504, p-value=0.023).



[Table/Fig-5]: ROC curve for optimum cut-off of Ki-67 for complete pathological response.

Ki-67 category	Pathological response, n (%)		Total, n (%)	Odds ratio	p-value
	pCR	Non pCR			
≥40%	6 (75)	12 (28.6)	18 (36)	1.000 (Ref.)	
<40%	2 (25)	30 (71.4)	32 (64)	7.50	**0.023**
Total	8 (100)	42 (100)	50 (100)	-	

[Table/Fig-6]: Association of Ki-67 (cut-off categories) with pathological response. The p-value in bold font indicates statistcially significant value

DISCUSSION

Pathologic complete response is the gold standard for assessing the efficacy of NAC in breast cancer [14]. Biomarker testing enables categorisation of carcinomas into luminal subtypes and the assessement of disease aggressiveness is possible with Ki-67 testing with suggested threshold of 14-15%, to discriminate between cases which likely correlate with more aggressive luminal B cell type with Ki-67 >14 or 15% and luminal A with Ki-67 <14 or 15% [11]. However, there is no set cut-off for Ki-67 to predict pathological complete response. A total of 50 cases of breast cancer were evaluated in the present study. The mean±SD age of patients was 53.3±10.3 years, which is comparable to the study conducted by Stamatovic L et al., where the mean age of 190 patients who were analysed for response to NAC, was 52 years [15]. In the present study, 8 (16%) patients had achieved pCR, while 18 (36%) patients showed pPR and 24 (48%) had pNR. While the studies done by McFarland DC et al., and Mancinelli BC et al., showed overall pCR rate was 26.5% and 21.6%, respectively, suggesting multifactorial attributes such as luminal subtypes to pCR [16,17]. Mean value Ki-67 in pCR was 56.25%, pPR was 36.75 and in pNR was 29.5%. Ki-67 and pathological response showed statistically significant association (p-value=0.03). Statistically significant difference was present between pCR and non pCR (p-value=0.027).

Fasching PA et al., studied 552 patients who underwent NAC and found Ki-67 to be an independent predictor for pCR and for overall survival and distant disease-free survival. Patients with pCR and non pCR showed a mean Ki-67 value of 50.6±23.4% and 26.7±22.9% respectively and thus concluded that Ki-67 has predictive and prognostic value in post chemotherapy breast carcinoma [18].

Sueta A et al., evaluated pretherapeutic Ki-67 in 121 breast cancer core biopsies and found that Ki-67 is an independent prognostic marker especially in ER positive cases, and stratification according to Ki-67 levels might improve predictive significance of the response in hormone-responsive breast cancer [19]. Schlotter CM et al., also suggested that pCR rates were higher in tumours with higher proliferation [20]. Optimal percentage cut-off for Ki-67 in initial trucut biopsy of breast that could effectively predict complete pathological response was found to be 40%. Six cases with Ki-67 >40% was associated pCR (75%) and the association was statistically significant (p-value <0.05). The study by Ács B et al., suggested NAC was more efficient in tumours with atleast 20% Ki-67 [21], while Resende U et al., suggested that Ki-67 ≥50% expression were independent predictors to confirm pCR [8].

Limitation(s)

The present study needs to be extrapolated to a large population-based sample which may improve the specificity and sensitivity of the Ki-67 percentage cut-off value.

CONCLUSION(S)

A significant link has been established between Ki-67 score and pathological response through the present study. The Ki-67 percentage cut-off that was found to be attributable to complete pathological response was 40%. From the present study, it can be effectively concluded that Ki-67 can be used as an independent predictive marker of pathological response in patients undergoing NAC. In the future, new developments in Ki-67 standardisation are possible, which can increase the utility of Ki-67 as a prognostic marker. Use of automated software-based Ki-67 counting index and artificial intelligence assisted Ki-67 analysis can reduce manual error and enhance score reproducibility.

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