RESEARCH ARTICLE

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Association of Proliferative Activity in Invasive Ductal Carcinoma Breast in Pakistani Population

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Abstract

Background: Personalized medicine has played very important role in management of breast cancer. Proliferative index is one among the prognostic and predictive factor but unfortunately due to varied reports , no definite consensus and routine medical practice has been approved for it. The objective of the study is to observe the association of Ki-67 index using St. Gallen Conference criteria in invasive ductal carcinoma breast in Pakistani Population. **Methods:** Eighty-three patients with confirmed light microscopic diagnosis of primary invasive ductal carcinoma were recruited in this prospective study . Expression of Ki67 was determined by classifying as low (<15%) and high (>15%) Ki67 in tumour. Statistical analysis was performed to observe the association of Ki-67 with clinicopathological parameters and molecular group (i.e., Luminal A, Luminal B, Her2 enriched and triple negative). **Results:** Out of 83 patients, 73.5% of patients showed >15% Ki67 (p value <0.001). High expression of Ki 67 (>15%) was observed in 3.6%, 21.7% and 48.2 % of Nottingham grade I, II and III (p value=0.017) respectively. Among molecular group, high expression of Ki67 was observed 20.5% in Luminal A, 9.6% in Luminal B, 15.7% in Her2 enriched and 27.7% in triple negative groups (p= 0.017). There was no significant association observed in expression of Ki 67 among lymph node stage, tumour stage and Nottingham prognostic index. **Conclusion:** Higher Ki-67 reactivity is usually associated with higher-grade morphology of tumour. It can act as an independent predictor in assessment of tumour behavior. However, larger validation clinical studies are still required for confirmation of its importance and for routine practice.

Keywords: Breast carcinoma- Ki-67 antigen- prognosis- tumour grading- triple-negative breast cancer

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Introduction

Breast carcinoma is considered as a heterogeneous disease. The phenotypic diversity significantly affects the diagnosis and prognosis. Female breast cancer has become the most common tumour among new cases in year 2020 with incidence rate of 88% higher in transitioned countries as compared to transitioning countries (Sung et al., 2021). In Pakistan, nearly 1 in 9 patients has lifetime risk of development of breast cancer, which is 2.5 times higher than that of neighbouring countries and also high when compared to Western population (Zaheer et al., 2019; Asif et al., 2014). Similar to many developing countries, in Pakistan, the cost/expenditure of entire management of carcinoma are being borne by the patients, which imposes a heavy financial burden on them.

Proliferative activity of tumour is considered as one of the predictor of biological behaviour, response to chemotherapy and treatment effectiveness along with recurrence risk of the tumour. Ki-67 is an immunologic nuclear proliferative nuclear antigen expressed in different phases i.e., S, G1, G2 and M stages of cell cycle (Varga et al., 2017; Inwald et al., 2013; Shandiz et al., 2016). Ki-67 expression carries valuable prognostic information and has probably a predictive effect in lymph node negative breast cancer patients (Shandiz et al., 2016). Its assessment can help physician in treatment selection. Ki-67 index can be useful in classifying grade two-breast cancer into low and high-risk subgroups. The standardization of Ki-67 assessment has been most intricate and challenging. There are many factors, which affects its estimation including objective changeability in selecting tumour areas of assessment, technique of performing immunohistochemistry, conundrum of cut-off and counting of expression of nuclear positivity particularly in larger tissue sections, type of fixative, time to fixation, means of storage, antigen retrieval and counterstain. Method of reading, area of slide read affects

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Ki-67 immunohistochemistry (Varga et al., 2017; Dowsett et al., 2011). Neither in the St. Gallen Conference, nor in the ASCO recommendations Ki-67 was advocated for routine use, despite of knowing the potential of Ki-67 involving prognosis, prediction of relative response or deficiency to chemotherapy and as a dynamic biomarker of the treatment effectiveness (Inwald et al., 2013). Higher rates of proliferation are most likely to be benefitted from chemotherapy. Ki-67 has been chosen to be the primary end point, replacing the conventional clinical endpoint of tumour shrinkage (Dowsett et al., 2011).

In the present study, we intend to evaluate the association of Ki-67 reactivity with various clinicopathological parameters and molecular classification of invasive ductal carcinoma cases.

Materials and Methods

Subject and Method

This is a prospective study comprised of 83 mastectomy/ modified mastectomy specimens, microscopically confirmed invasive ductal carcinoma patients from two tertiary care hospitals of Lahore (Mayo Hospital & Shalamar Hospital, Lahore, Pakistan). The cases who received neoadjuvant therapy were excluded. The study was approved as part of Ph.D research by advanced studies & Research Board, University of Health Science. Pakistan (# UHS/Education/126-16/215). After routine tissue processing and Hematoxylin & Eosin staining, Nottingham histological grading was done. Based of Nottingham Prognostic Index (NPI) was calculated as: (0.2x tumour diameter in cm) + lymph node stage + tumour grade (Ring et al., 2006). The prognostic categories used for NPI: Good outcome less than 3.4≤moderate risk ≤ 5.4 less than poor expected outcome.

Two representative paraffin embedded sections of tumours cut at 4 µm were selected for Ki-67 immunohistochemical staining. Primary antibody of Ki67 as Monoclonal Mouse Anti Human by Dako Cytomotion (ready to use, code IS626) with positive and negative controls along with mouse and rabbit specific HRP Plus (ABC) detection IHC kit (Ready to use, # ab93697) was used as per instruction manuals. At least 500-tumour cells were counted manually for assessment of nuclear positively stained tumour cells. A Ki-67 cut-off point of 15 % was defined (Inwald et al., 2013) and cases were classified as low proliferative (low Ki-67 <15%) and high proliferative (high Ki-67 >15%) tumours for evaluation of clinico pathological data was collected and molecular classification of breast cancer. Slides were examined and evaluated by three-consultant histopathologist independently. In case of difference of opinion, two similar results out of the three were considered final. The data was entered and analysed using IBM SPSS version 27. Frequencies and percentages were reported for qualitative variables. Pearson Chi-square and Fisher Exact tests were applied to observe associations between qualitative variables. A p-value of less than 0.05 was considered as statistically significant.

Results

Eighty-three invasive ductal carcinoma patients were recruited in this study after informed consent. Patients' ≤ 50 years of age were 57 (69%) and above

Table 1. Shows the Low and High Expression of Ki-67 in AAssociation with Clinicopathological Parameters, Nottingham Prognostic Index and Molecular Classification

	Ki-67 < 15% n (%)	Ki-67 >15% n (%)	P- value
Total patients, n = 83	22 (26.5)	61 (73.5)	<0.001*
Age			0.457
≤50 years	14 (16.9)	44 (53)	
>50 years	8 (9.6)	17 (20.5)	
Glandular component			0.007*
>75%	0	1 (1.2)	
10-75%	14 (16.9)	16 (19.3)	
<10%	8 (9.6)	44 (53)	
Nuclear grade			0.15
Mild	1 (1.2)	0	
Moderate	14 (16.9)	33 (39.8)	
Severe	7 (8.4)	28 (33.7)	
Mitotic count	22	61	0.018*
0-9	0	3 (3.6)	
10-19	17 (20.5)	26 (31.3)	
>19	5 (6)	32 (38.6)	
Nottingham grade			0.017*
I	1 (1.2)	3 (3.6)	
II	14(16.9)	18 (21.7)	
III	7 (8.4)	40 (48.2)	
N stage			0.117
N0	10 (12)	14(16.9)	
N1	4 (4.8)	25 (30.1)	
N2	5 (6)	17 (20.5)	
N3	3 (3.6)	5 (6)	
Tumour stage			0.171
T2	14 (16.9)	26 (31.3)	
T3	6 (7.2)	20 (24.1)	
T4	2 (2.4)	15 (18.1)	
NPI			0.348
Good	2 (2.4)	3 (3.6)	
Moderate	9 (10.8)	17 (20.5)	
Poor	11(13.3)	41 (49.4)	
Estrogen receptor			0.002*
Positive	18 (21.7)	27 (32.5)	
Negative	4 (4.8)	34 (41)	
Progesterone receptor			0.001*
Positive	18 (21.7)	25 (30.1)	
Negative	4 (4.8)	36 (43.4)	
Molecular classification			0.017*
Luminal A	14 (16.9)	17 (20.5)	
Luminal B	2 (2.4)	8 (9.6)	
Enriched (Her2 +)	4 (4.8)	13 (15.7)	
Triple negative (TN)	2 (2.4)	23 (27.7)	

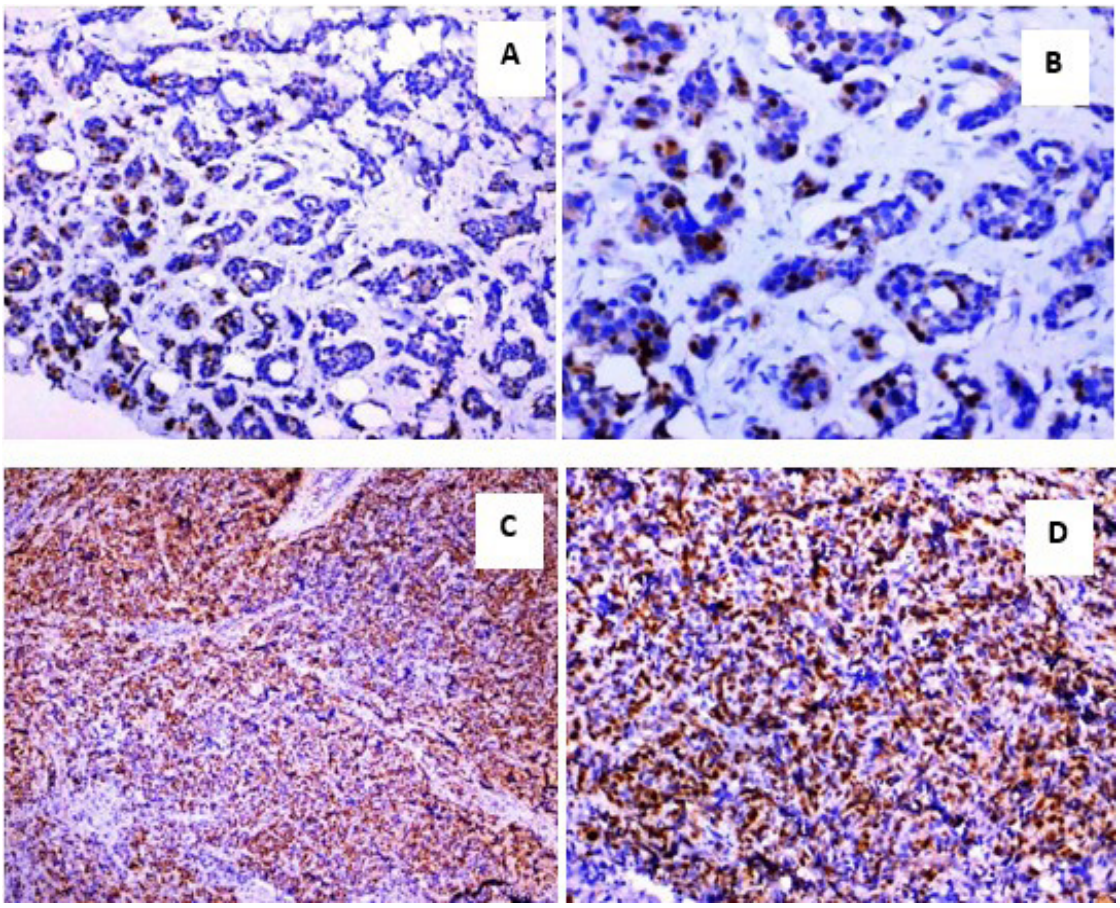

Figure 1. A, Microphotograph shows low (<15%) Ki-67 reactivity (100x); B, Low (<15%) Ki-67 reactivity (200x); C, High (>15%) Ki-67 reactivity (40x); D, High (>15%) Ki-67 reactivity (100x).

50 years of age were 27 (21.3%). Glandular component formation less than 10 % formation, >19 mitosis/HPF and moderate nuclear pleomorphism showed highest number of patients with high Ki67 expression in 53%, 38.6% & 39.8% of cases. High expression of Ki-67 statistically significantly associated (p value < 0.05) with poor glandular formation, high mitotic count and so poorly differentiated invasive ductal carcinoma. Poor NPI group showed high expression of Ki-67 as compared to moderate and good outcome groups. Negative estrogen and progesterone receptor expression showed high Ki-67 expression in 41% and 43.4% of patients & is significantly associated (p <0.05). In luminal A, B, Enriched and triple negative groups, low Ki-67 was found in 16.9%, 2.4%, 4.8% and 2.4%, whereas, high Ki-67 was observed in 20.5%, 9.6%, 15.7% and 27.7% of patients respectively. Summarizing, significant association of Ki-67 was found in glandular component (score 3, p = 0.007), mitosis (score 3, p = 0.018), Nottingham grade (III, p = 0.017), negative hormone receptor (p<0.05), triple negative (p=0.008). No association was observed with age (p = 0.457), lymph node stage (p= 0.117), tumour stage (p= 0.171), nuclear grade (p= 0.150), Her2 reactivity (p= 0.295) and NPI (p= 0.348).

Discussion

International Ki-67 in Breast cancer Working group in 2019 accepted Ki67 as a prognostic marker with clinical validity but its utility is evident only for estrogen receptor positive and Her2 negative patients who do not need adjuvant chemotherapy. Results of Ki-67 can be affected by preanalytical process (e.g., fixation time, thickness of section), analytical factors (including antigen retrieval, counterstain etc) and interpretation and scoring (e.g., manual and digital imaging) (Nielsen et al., 2020). Ki-67 is nuclear protein, used for proliferation assessment of cellular proliferation, expressed in all stages of cell cycle with the exception of G0. Higher Ki-67 expression is associated with higher risk of relapse and worse survival in early breast cancer patients (Zeng et al., 2019). A review by Mannell (2016) on role of Ki-67 in breast carcinoma stated that aggressive behavior of tumour, including negative hormone receptor, higher tumour grade, positive nodal status, young age and lymphovascular invasion, had been associated with worse outcome. High percentage of Ki-67 remained an independent prognostic parameter for disease free-survival and overall survival rate. Ki-67 value could predict neoadjuvant chemotherapy response (Ingolf et al., 2014).

Findings of the current study are consistent with most of the studies available in literature. Zeng et al., (2020) retrieved results as 54.5% showed high & 45.5% showed low Ki-67 expression with statistically significant association with luminal like group, however, no association with Her2 & TNBC group was recognized

(Zeng et al., 2019). In TNBC, prognostic importance was weaker in this group (Zeng et al., 2019). They could not make out any significant association with Recurrence free survival in grade 3 patients, whereas, identified it as independent factor for grade 1 and 2.

Turkish study, Kanyilmaz et al., (2019) study group comprised of 258 patients including modified radical mastectomy and breast conservative surgery specimen, scoring was done with 3 tire system as 50 % of patients to express high Ki-67, 32% were intermediate and 18% with low Ki-67. They spotted significant association with tumour grade, lymph node stage, and hormone receptor.

Soliman and Yussif (2016) conducted a study on 107 primary breast cancer patients in Egypt using tissue microarray and differentiated 66.2% with low Ki-67 and 33.8 % with high Ki67. They reported significant association of Ki 67 with tumour grade and mitotic count among clinico-pathological characteristics (Soliman and Yussif. 2016). Among molecular subtypes, they detected significant association with hormone receptor positivity (Soliman and Yussif. 2016). All patients (100%) in Luminal A group showed low Ki-67, 70% of luminal B group showed Low Ki-67 and 60% of triple negative patients showed high Ki-67 with statistically significant association. However, no association was noticed with Her2neu receptor, 14 probably because of less patients in this group.

Alco et al., (2015) determined 60.8% with high Ki-67 and 39.2% with low Ki-67. They observed negative correlation between high Ki-67 and negative hormone receptor and positive correlation with tumour size. More than 15% cut off for Ki-67 was found to be associated with most clinico-pathological prognostic factors. An Iranian study conducted on 220 patients, could not appreciated any correlation with patient age, tumour size, grade; marginal correlation with lymph node status, however, stated significant correlation between hormone receptor and Ki-67 (Kermani et al., 2019).

In conclusion, We concluded that high proliferative activity is likely be associated with poor outcome and high-grade tumours. Since in the present study, the number of patients were less, validation studies are required to see and confirm expression of Ki-67 as a independent prognostic and predictive factor, which will likely help the physicians to plan the management plan for the patients.

Author Contribution Statement

NH: Concept and design of study, acquisition of data, drafting and critical input in revising manuscript. NH, AR, FBN : Acquisition of data, drafting and revision of the manuscript with intellectual input. WS: Analysis of data. UA: Critical Revision and final revision and drafting of manuscript. ALL AUTHORS: Approval of the final version of the manuscript to be published.

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