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Original Article

Correlation of Percentage of Ki67 with Tumor Grade in Breast Cancer

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ABSTRACT

Objective: To determine the association of Ki67 with tumor grade and other pathological features in patients with breast carcinoma.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Department of Medical Oncology of Jinnah Postgraduate Medical Center, Karachi Pakistan, from Jul 2018 to Jul 2019

Methodology: Two hundred and sixty females diagnosed with breast carcinoma of age more than 19 years were included using non-probability consecutive sampling technique. The histologic grade of tumor has been assessed using the classification of the World health organization. Representative sample was selected from each tumor for immune histochemical staining examination including estrogen receptors, progesterone receptors, Her2neu and Ki67. Nuclear expression has been quantitatively documented for Ki67.

Results: The mean age of the study sample was noted as 46.93±12.8 years ranging from 20 to 76 years. Of 260 patients, 17 patients (6.5%) had low Ki67 (1-5%), 23 patients (8.8%) had intermediate Ki67 (6-14%) and 220 patients (84.6%) had high Ki67 (≥15%). The significant relationship was found between Ki67 status and age, stage, tumor size, grade, lymph node status, estrogen receptors, progesterone receptors, HER2/Neu and triple negative (p<0.05).

Conclusion: Ki67 index is associated with tumor grade and other pathological features such as stage, tumor size, lymph node status, estrogen receptors, progesterone receptors, HER2/Neu and triple negative.

Keywords: Breast carcinoma, Estrogen receptors, Ki67 index, Lymph node status, Progesterone receptors, Tumor grade, Tumor size.

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INTRODUCTION

Globally, the second most common cause of death among females is breast carcinoma. Worldwide about 2.08 million new cases of breast cancer reported in 2018, comprising 24.2% of all cancer cases. The incidence of breast cancer and death rates in middle and low-income countries has risen significantly over the past decades.[1] Pakistani young women are more affected with breast cancer than Western women. According to recent research, in Pakistan 34,038-90,000 new cases of breast cancer and 16,232 deaths related to breast cancer have been occurred in 2017.[2]

Knowing prognostic factors are important for breast cancer treatment selection for whom adjuvant therapy can improve the prognosis. Conventional prognostic variables such as cancer type, grade, presence of the lymph node and surgical margin can only describe the category and result of about 30% of patients. Hence, new markers of prognosis are therefore required.[3-5] Given that radiation therapy and various medical hormone manipulations may cause side effects in patients, treatment strategies based on risk factors are important for minimizing such side effects. Over the past few years, different prognostic factors have been identified. Most of them, though, do need medical clinical validation.[6]

When investigating possible breast cancer prognostic factors, research has been centered on tumor markers. In the clinical behavior of invasive breast cancer the cell proliferation plays a vital part. Ki67 is a nuclear receptor present in proliferative cells. Several trials have shown that Ki67's immune response is closely related to the process of cells.[5,7,8] In fact, Ki67 can influence the frequency of relapse after successful treatment in patients with carcinoma of breast and raised Ki67 after neoadjuvant chemotherapy is associated with poor prognosis.[5,8,9]

Ki-67 immuno-histochemistry (IHC) is an inexpensive and easy technique that can be readily used in virtually all pathological laboratories and needs only a small sample of tissue, including those collected from fine-needle aspirations (FNA). The goal of the current research was to determine the

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correlation of Ki67 with tumor grade and other clinicopathological features in patients with breast carcinoma. This research will help stratify patients into prognostic subgroups with a stronger predictive response to hormonal or chemotherapy adjuvant or neoadjuvant.

METHODOLOGY

It was a cross-sectional analytical study conducted at the department of Medical Oncology of Jinnah Postgraduate Medical Center, Karachi Pakistan, from Jul 2018 to Jul 2019. Sample size was estimated using proportion of Ki67 (15–24%) in Pakistan as 18.3%,[10] among infiltrating ductal carcinoma, absolute precision as 4.5% and 95% confidence level, the calculated sample size came out as 284.

Inclusion Criteria: All females diagnosed with breast carcinoma of age more than 19 years were included using non-probability consecutive sampling technique.

Exclsuion Criteria: Non-epithelial tumors and patients with post-chemotherapy were excluded.

Approval of the ethics review committee (ERB NO.F.2-81/2018-genl/7069/JPMC) was obtained prior to the start of study. Informed consent was taken from all the eligible patients. The histologic form of tumor has been assessed using the classification of the WHO.[11] Representative sample was selected from each tumor for immuno histochemical staining (IHC) examination including PR, ER, Her2neu and Ki67. Antibodies for ER, PR and Her2neu IHC were extracted from DAKO and DAKO envision kit was used and stains were produced in keeping with the manufacturer's defined procedure. Every IHC test was conducted with negative and positive controls. Only semi-quantitative nuclear expression of ER and PR was recorded and as an optimistic expression more than 1 percent of expression was taken.[12,13] For Her2neu IHC, only membranous staining was observed and as per CAP guidelines, more than 10% heavy membranous positivity was taken as favorable (3+) Her2neu IHC.[14,15] Nuclear expression has been quantitatively documented for Ki67. To calculate an average estimate, at least 1000 cells were evaluated. Ki67 index was further classified into three categories based on the percentage of staining, 1-5%, 6-14%, and ≥15%.[5]

SPSS version 23 was used to analyze data. Mean±SD was reported for numeric variables. Frequency and percentage was reported for categorical and nominal variables. As the outcome variable i.e. Ki67 status was ordinal therefore linear by linear association was used to see the significance between Ki67 and effect modifiers. The *p*-value ≤0.05 was taken as statistically significant.

RESULTS

Total 284 cases were selected but after excluding incomplete and missing data 260 females were included in the study. The mean age of the study sample was noted as 46.93±12.8 years ranging from 20-76 years. Out of 260 patients, majority of patients has T2 tumor size 145(55.8%) followed by T1 59(22.7%) and T3 53(20.4%). Eighty two patients had histological grade 3, 157 patients had grade 2 and 21 patients had grade 1 respectively. IDC was the most frequent histological subtype including 233 cases, followed by ILC accounting for 25 cases. Approximately 46.2% of the cases were in stage II of tumor and 241 cases were lymph node positive (N1-N3). Of all patients, 171(65.8%), 176(67.7%), and 145(55.8%) were ER positive, PR positive, and HER2/Neu positive, respectively. (Table-I)

Table-I: Characteristic Of Study Variables (n=260)

Variables	Mean±SD
Age in years	46.93±12.8
	n(%)
Histological Grade	
I	21(8.1)
II	157(60.4)
III	82(31.5)
Histological Subtype	
Invasive ductal carcinoma (IDC)	233(89.6)
Invasive lobular carcinoma (ILC)	25(9.6)
Mucinous carcinoma	1(0.4)
Leiomyosarcoma	1(0.4)
Stage of Tumor	
I	35(13.5)
II	120(46.2)
III	69(26.5)
IV	36(13.8)
Tumor Size	
T1	59(92.27)
T2	145(55.8)
T3	53(20.4)
TIV	3(1.2)
Lymph Node Status	
No	19(7.3)
N1	106(40.8)
NII	116(44.6)
NIII	19(7.3)
ER	
Positive	171(65.8)
Negative	89(34.2)
PR	
Positive	176(67.7)
Negative	84(32.3)
HER2/Neu	
Positive	145(55.8)
Negative	115(44.2)

The mean Ki67 index was reported as 33.11±20.84. Of 260 patients, 17 patients (6.5%) had low Ki67 (1-5%), 23 patients (8.8%) had intermediate Ki67 (6-14%) and 220 patients (84.6%) had high Ki67 (≥15%). Among 120 patients with stage II of tumor, majority of the patients (80%) had high Ki67, followed by intermediate Ki67 (12.5%) and low Ki67 (7.5%). The significant relationship was found between Ki67 status and stage of tumor by applying chi-square test (p=0.001).

Among 157 patients with histological Grade-II, 87.9% had high Ki67 (≥15%), 6.4% had low Ki67(1-5%) and only 5.7% had intermediate Ki67(6-14%). The significant association was found between Ki67 status and tumor grade (p=0.043) by applying chi-square test.

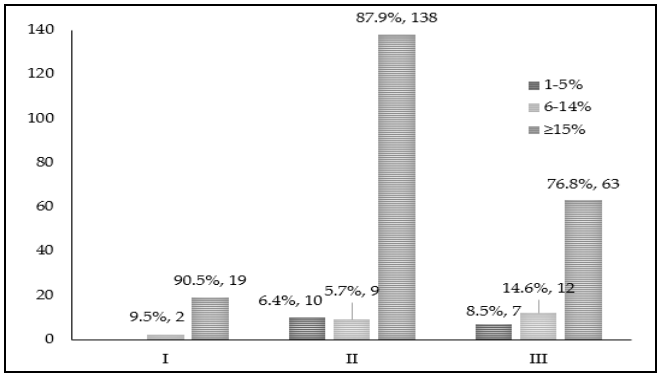

Figure-1: Relationship of Ki67 with Tumor Grade (n=260)

About 7 patients who were triple negative had high Ki67 (≥15%), 6 had intermediate Ki67(6-14%) and 5 had low Ki67(1-5%). The statistically significant association was found between triple negative and Ki67 status (p=0.001) by applying chi-square test.

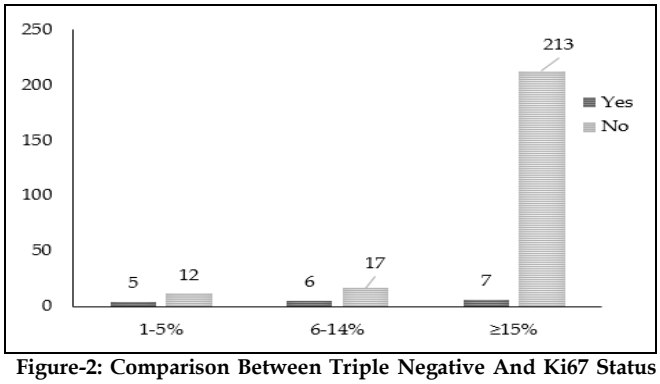

Figure-2: Comparison Between Triple Negative And Ki67 Status (n=260)

The significant relationship was also found between Ki67 status with age (p=0.001), lymph node status (p=0.001), ER (p=0.002), PR (p=0.001) and HER2/ Neu (p=0.001) by applying chi-square test.

Table-II: Association of Ki67 with Resect to Effect Modifiers (n=260)

Effect modifiers	Ki67 status			*p*-value (Chi-sq test)
	1-5%	6-14%	≥15%	
Age groups				
≤45 years	17(100%)	19(82.6%)	85(38.6%)	0.001
>45 years	0	4(17.4%)	135(61.4%)	
Histological subtype				
Invasive ductal carcinoma (IDC)	10(58.8%)	19(82.6%)	204(92.7%)	0.057
Invasive lobular carcinoma (ILC)	7(41.2%)	4(17.4%)	14(6.4%)	
Mucinous carcinoma	0	0	1(0.45%)	
Leiomyosarcoma	0	0	1(0.45%)	
Lymph node status				
No	4(23.5%)	7(30.4%)	8(3.6%)	0.001
N1	8(47.1%)	7(30.4%)	91(41.4%)	
N2	1(5.9%)	8(34.8%)	107(48.6%)	
N3	4(23.5%)	1(4.3%)	14(6.4%)	
Estrogen receptors (ER)				
Positive	6(35.3%)	12(52.2%)	153(69.5%)	0.002
Negative	11(64.7%)	11(47.8%)	67(30.5%)	
Progesterone receptors (PR)				
Positive	2(11.8%)	10(43.5%)	164(74.5%)	0.001
Negative	15(88.2%)	13(56.5%)	56(25.5%)	
HER2/Neu				
Positive	5(29.4%)	6(26.1%)	134(60.9%)	0.001
Negative	12(70.6%)	17(73.9%)	86(39.1%)	

DISCUSSION

Around the world, invasive breast cancer is the prevalent malignant tumor among women and is a heterogeneous cancer with distinct clinical and pathological features that can be divided into several subtypes dependent on the expression of three receptors: ER, PR and HER2. Ki67 is an immuno-histochemical precursor for proliferation in many cancer types and has been extensively studied in breast cancer patients for the assessment of relapse and survival of breast cancer patients, mostly through retrospective research.[8] In the current research we have evaluated the association of Ki67 percentage with tumor grade and other pathological factors among breast cancer patients.

The suitable cut-off value for Ki67 is still arguable for oncologists and widely examined.[16-18] Because of specific patient populations in each series, the use of data-derived 'optimal ' cut-points may result in serious bias. It should be stressed that the conversion of continuous variables such as the Ki67 index into two classes may result in the biomarker's power loss.[19] Furthermore, this is impractical at the individual level, as it implies that patients who have tumors with Ki67 rates near to the cut-point but on either side of the cut-

point are very unique, and in effect receive different treatments, whereas in fact they are likely to be very identical. Few authors specifically focused their study on Ki67 cut-off values, but failed to identify a single optimal value when showing a consistent correlation between increasing numbers of stains and worse outcomes.[10,20]

As proposed by the St. Gallen International Expert Consensus, they followed the cut-off points at 15% and 30% and graded the Ki67 into low, intermediate and high-risk categories. This method is particularly useful because it reveals a core gray zone between low and high Ki67 levels, while other variables are used to render treatment decisions.[10,21,22] Whereas, in a latest research by Kermani *et al.* classified Ki67 index into three categories based on the percentage of staining, 1-5% as low, 6-14% as intermediate and ≥15% as high.[5] We adopted Kermani *et al*. criteria in current research and found majority of the patients were in high (84.6%) Ki67 category. In the research by Haroon *et al.* also found more than half of the patients had cut off value for Ki67 as more than 15%, wherein 27.8% of the patients were in intermediate and 31.9% were in high risk groups.[10] In the research by Soliman *et al*. dissimilar results was found, 36 patients had Ki67 value more than 15% and 71 patients had Ki67 value less than 15%.[7] Madani *et al*. in their research found 55.4% of the patients had low Ki67 (value <20%) and 44.6% of the patients had high Ki67 (value ≥20%).[8] Nevertheless, it is well known that higher levels of the Ki67 proliferation predictors are associated with increased recurrence, poor survival and high mortality rates.[17,18]

In the present research, tumor size was positively associated with Ki67 status (p<0.05). Almost 55% of the patients with had high Ki67 had T2 tumor size. Marwah *et al.* in their research observed majority of the patients with tumor size 2-5cm had high Ki67 positivity and showed statistically significant relation.[23] In the study by Ragab *et al*. found 79 patients had tumor size= >2cm, where in 56 patients had Ki67 >20%. In another study by Soliman *et al.* found majority of the patients had tumor size >2cm and insignificant association between tumor size and Ki67 positivity.[7] Madani *et al.* also found no significant association between tumor size and Ki67 status.[8] In the Pakistani research by Saroon *et al.* observed 65 patients had tumor size T2 and 31 patients had tumor size T3 and among them majority of the patients had low Ki67, hence no significant association was found between tumor size and Ki67 status.[10]

In the present study, significant difference was between categories of Ki67 and lymph node status and stage of tumor. Almost 120 patients had stage two tumor where in majority of them had high Ki67 value greater than 15% and more than 7% of the patients had high Ki67% who significant lymph node involvement. In the study by Soliman *et al.* found majority of the patients had stage 3 tumor (53.3%) where in most of them had Ki67 value<15% and no significant relationship was found between tumor stage and Ki67 status and 83% patients who showed positive lymph node had high Ki67, however no statistically significant association was found.[7]

In the present research, majority of the patients were of age more than 45 years (53.5%) and among them majority (61.4%) had significantly high Ki67 status (>15%). Soliman *et al*. found in their research majority of the patients of age more than 55 years and among them 31% had Ki67 expression (>15%) whereas no significant association was found between age and Ki67 expression.[7] In the present research, Ki67 status was positively associated with ER, PR, HER/Neu2 and triple negative breast cancer with p-values <0.05 respectively. Altintas *et al.* found high Ki67 proliferative lesions were more likely to be PR negative and ER negative.[24] In another study by Kermani *et al.* found a significant and marginally significant correlation of Ki67 with ER and PR.[5] In the study by Saroon *et al.* significant association was found in Ki67 expression with PR and HER/Neu2 status.[10] Soliman *et al*. found Ki67 expression (>15%) was negatively associated with ER, about 60% of the patients with triple negative breast cancer had significantly high value of Ki67 (>15%), whereas no significant association was found between HER/Neu2 and Ki67 expression.[7]

CONCLUSION

Ki67 index is associated with tumor grade and other pathological features such as stage, tumor size, lymph node status, estrogen receptors, progesterone receptors, HER2/Neu and triple negative.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

AY: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

GH & SZ: Data acquisition, data analysis, approval of the final version to be published.

MN & NB: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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