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Expression of Ki-67 in Patients of Invasive Ductal Carcinoma and its Correlation with Histological Grades

Yusra Saleem[1], Rabia Ahmed[2], Hina Wasti[3], Nighat Jamal[4], Hira Faisal[5], Shaista Bakhat[6]

1 *Classified (Histopathologist), PNS Shifa Hospital, Karachi Pakistan*
Contribution in the study

2 *Surg Lt Cdr, Assistant Professor, PNS Shifa Hospital Karachi Pakistan*
Contribution in the study

3 *Assistant Professor, Department of Histopathology, Bahria University Health Sciences, Karachi Pakistan*
Contribution in the study

4 *Surg Cdr, Associate Professor Histopathology, PNS Shifa Hospital Karachi Pakistan*
Contribution in the study

5 *Senior Lecturer, Department of Histopathology, Bahria University Health Sciences, Karachi Pakistan*
Contribution in the study

6 *Assistant Professor, Department of Microbiology, Bahria University Health Sciences, Karachi Pakistan*
Contribution in the study

CORRESPONDING AUTHOR
Dr. Hina Wasti
Assistant Professor, Department of Histopathology, Bahria University Health Sciences, Karachi Pakistan
Email: drhinafaisal@gmail.com

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ABSTRACT

Background: Histological grade which is an important determinant of breast cancer prognosis. It is evaluated by pathologists according to the Nottingham modification of the Scarff-Bloom-Richardson (SBR) grading system. It could be incorporated into algorithms to choose the most appropriate treatment for patients with breast cancer. Prognostic factors are important in breast cancer as they allow the identification of high-risk patients, for which, adjuvant therapy can improve the prognosis. Amongst the prognostic markers, the Ki67 index is considered a practical method for predicting the responsiveness to chemotherapy. **Objective:** To compare histological grades of carcinoma with Ki67 expression in patients of invasive ductal carcinoma breast. **Study Design:** Descriptive, cross-sectional. **Settings:** Department of Pathology, PNS SHIFA Hospital, Karachi. **Duration:** 13th February 2021 to 12th August 2021. **Methods:** 77 cases of invasive ductal carcinoma of both genders and ages ranging from 25-85 years were included. Histologic grading was performed on H&E stained sections using the Nottingham modification of the Scarff-Bloom-Richardson (SBR) grading system. Immunohistochemistry for Ki67 was done on FFPE tissues using the DAKO Envision kit. Ki67 the index was determined as the number of Ki67 positive cells per 500 tumor cells. Ki67 was graded as low <14% and high ≥14%. **Results:** In our study, histological grading of invasive ductal carcinoma was found to be as follows; grade I in 01 (1.30%), grade II in 53 (68.83%), and grade III in 23 (29.87%) patients. A high Ki67 index was found in 70 (90.91%) patients. All grade III tumors showed high expression of Ki67, while 88.68% of grade II tumors showed high expression. None of the grade I tumors showed high Ki67 expression. **Conclusion:** This study showed that grade II (75.71%) was the most common grade of invasive ductal carcinoma followed by grade III (22.86%). High expression of Ki67 correlates with a higher histological grade.

Keywords: *Histologic grade, Invasive ductal carcinoma, Ki67.*

INTRODUCTION

One of the most prevalent cancers and the leading cause of cancer death among females is breast cancer. It accounts for 25% of all cancer cases and 15% of all cancer deaths in women.[1] The incidence of breast cancer in females is 22.9%. About 18.2% of deaths occur due to breast cancer in both genders. In Pakistan, breast cancer incidence among females is 34.6% which is the highest among Asians, the second-highest in the world after Jews in Israel, and 2.5 times more than the neighboring countries. [3]There are several contributing factors in the development of breast cancer, like early age of menarche, late age at first pregnancy, lesser pregnancies, decreased or no breastfeeding, and late menopause. Other factors include hormone replacement therapy, alcohol consumption, and obesity.[4]

It is generally acknowledged that breast cancer is a heterogeneous disease with a wide spectrum of clinical, pathologic, and molecular features. For the diagnosis of breast cancer, certain biomarkers are routinely available like ER, PR, and HER2, which are reliable, inexpensive, and useful for therapeutic decision making and can be a

reasonable substitute for the more expensive molecular subtyping.[5,6]

An increasing number of studies have suggested that Ki67 may be an important factor in cancer grading and prognostic evaluation.[7] It has been shown that Ki67 immunohistochemical (IHC) staining is an effective method of assessing the prognosis in many tumor types.

Ki67 is a sensitive protein associated with cell proliferation. The prognostic value of Ki67 has been investigated in several studies with its potential as a reliable marker having been shown in cancers of the breast, soft tissue, lung, prostate, cervix, and central nervous system.[8,9,10]

The histologic grading of invasive ductal carcinoma was defined and graded based on three parameters, mitotic activity, nuclear pleomorphism, and the extent of tubule formation by microscopic examination of the slides.

Table 1: Histological grades and basic parameters

Tubule Formation	Score	Mitotic count	Score	Nuclear Pleomorphism	Score
>75%	1	Up to 7	1	Small, uniform cells	1
10-75%	2	8 – 14	2	A moderate increase in size, shape with visible nucleoli	2
<10%	3	>15	3	Marked variation, multiple nucleoli	3

Score 3-5: Grade I (Well differentiated), Score 6-7: Grade II (Moderately differentiated), Score 8-9: Grade III (poorly differentiated)

Expression of Ki67: It was defined as low expression <14% and high expression ≥14%, by evaluating the nuclear expression of Ki67 in 500 tumor cells.[11,12]

As mentioned above Ki67 being a predictive maker can help clinicians to determine treatment modality for breast carcinoma patients. This study aims to evaluate the histological grades and expression of Ki67 in invasive ductal carcinoma in our population and to compare it with national and international data.

METHODS

This descriptive, Cross-sectional study was conducted at Department of Histopathology, PNS SHIFA Hospital, Karachi Pakistan. The duration of study 13th February 2021 to 12th August 2021.

A sample size of 77 was calculated with the WHO calculator by taking 95% confidence interval, 7% margin of error and prevalence of invasive ductal carcinoma of the breast, grade I = 11% (from a Pakistani study). Non-probability, consecutive sampling technique was used.

Patients of Trucut, incisional or excisional biopsy, mastectomy, age 25 - 85years, both genders, cases diagnosed as invasive ductal carcinoma and cases with ≥500 tumor cells were included in the study.

Poorly fixed tissue, tumor with extensive necrosis, post chemotherapy tumors and metastatic tumors were excluded from the study.

After approval from the College of Physicians and Surgeons of Pakistan and an ethical permission certificate from the Institutional Ethical Committee, the patients who fulfilled the inclusion criteria were included in the study. Clinical information including age, gender, place of residence, socioeconomic status, BMI, family history of breast cancer, and tumor laterality was recorded. Hematoxylin and Eosin-stained slides were prepared from the FFPE tissues.

A microscopic examination was done by a histopathologist to determine the histologic type and grade of the tumor. Immunohistochemical staining for ER, PR, and HER2 status was performed using DAKO Envision kit as per manufacturer's guidelines. ER and PR were reported according to Allred criteria (Annexure B). HER2 was reported according to ASCO/CAP guidelines (Annexure C). Immunohistochemistry for Ki67 was done on FFPE tissues using the DAKO Envision kit. Ki67 index was determined as the number of cells showing nuclear expression of Ki67 per 500 tumor cells. Ki67 was graded as low <14% and high ≥14%.8 Results of ER, PR, HER2 and Ki 67 were entered in the patient's proforma.

Statistical analysis was done using SPSS version 23.0. Continuous variables including age and BMI were presented as mean ± standard deviation. Categorical variables like gender, socioeconomic status, place of residence, laterality, family history of breast cancer, histopathological tumor grade, the status of ER, PR, HER2 and Ki67 grade were presented as frequency and percentage. Tumor grading and Ki67 grade were compared by using Chi-square test. Post-stratification Chi-square test was used. A p-value of ≤0.05 was taken as statistically significant.

RESULTS

The age range in this study was from 25 to 85 years with a mean age of 50.89 ± 14.14 years. The majority of the patients 47 (61.04%) were >55 years of age. The majority of the patients were females (97.4%). In our study, histological grading of invasive ductal carcinoma was found to be as follows; Grade I in 01 (1.30%), Grade II in 53 (68.83%), and Grade III in 23 (29.87%) patients.

High expression of Ki67 was found in 70 (90.91%) patients (Figure I). Expression of Ki67 was high in 0.0% of grade I, 88.68% of grade II and 100.0% of grade III invasive ductal carcinomas as shown in Table 2.

Table 2: Comparison of histological grade of invasive ductal carcinoma with Ki67 in patients of invasive ductal carcinoma

Histologic grade	Ki67		p-value
	High	Low	
I	00 (0.0%)	01 (100.0%)	0.002
II	47 (88.68%)	06 (11.32%)	
III	23 (100.0%)	00 (0.0%)	

Figure 1:

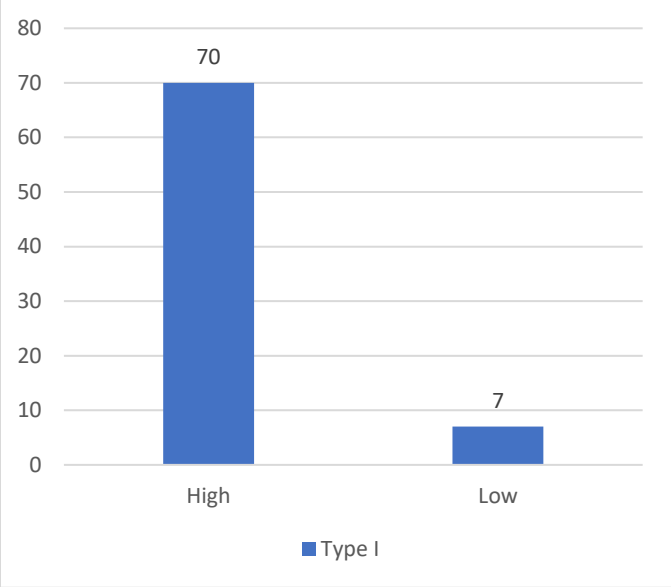


Figure 2: High ki-67 index

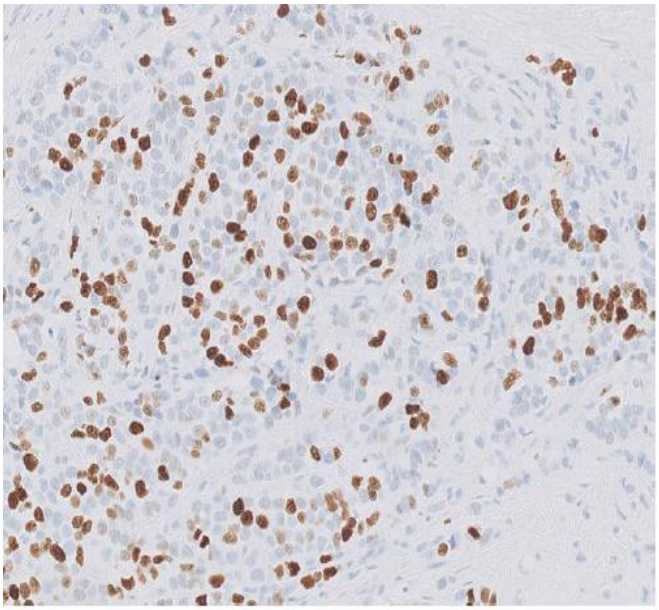
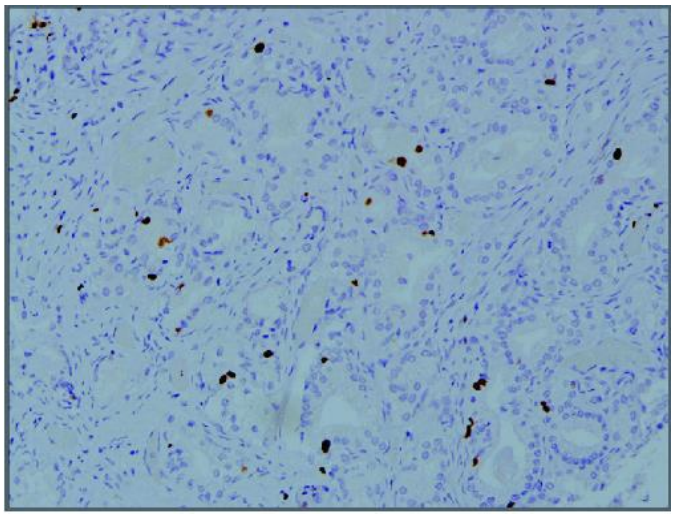


Figure 3: Low kI-67 index



DISCUSSION

The histological grade is an important determinant of breast cancer prognostication and could be incorporated into staging systems and algorithms to choose the most appropriate treatment for patients with breast cancer.[13] Histological grade is evaluated by breast pathologists according to the Nottingham modification of the Scarff-Bloom-Richardson (SBR) grading system. The tumor grade is determined by assessing morphological features (tubule formation, nuclear pleomorphism, and mitotic count). Therefore, the tumoral grade may be indirectly related to Ki-67 expression based on the mitotic count. However, few reports have described the correlation between Ki-67 and histological grade and how these two predictive factors are associated with the outcome of patients with different IHC-based subtypes of breast cancer.

We have conducted this study to determine the histological grading of invasive ductal carcinoma and to compare histological grades of invasive ductal carcinoma with Ki67 in patients of invasive ductal carcinoma. In our study, histological grading of invasive ductal carcinoma was found to be as follows; grade I in 01 (1.30%), grade II in 53 (68.83%), and grade III in 23 (29.87%) patients. The frequency of Ki67 high expression among patients with carcinoma breast is found in 70 (90.91%) patients. High Ki67 is found in 0.0% in grade I, 88.68% in grade II, and 100.0% in grade III. According to a cross-sectional and descriptive study on 100 cases of breast cancer in Dehradun, India 2013-2014, 64 patients were from invasive ductal carcinoma out of which a high Ki67 index (>30%) was seen in grade III IDC (89.4%), followed by grade II IDC (65.6%) and least number of patients of grade I IDC (45.4%) had high Ki67 index. [14]

A similar correlation between Ki67 and Nottingham grades was seen in a study on 125 patients with Invasive

ductal carcinoma at Central Laboratory. Abidjan, Ivory Coast. It concluded Ki67 as 40.3% in grade I, 56.58% in grade II, and 71.2% in grade III.[15]

Munzone et al analyzed 496 node-negative TNBC patients, with a mean age of 52 108 years and a median Ki-67 level of 48% (range, 4–95%). The study revealed that the Ki-67 index increased with decreasing age and increasing tumor size and grade. Furthermore, the Ki-67 level was significantly higher in the ductal TNBC cases, compared with the other histological types. Nishimura et al analyzed 2,638 BC patients, with mean age, tumor diameter, and Ki-67 value of 109 of 52.2 years, 2.2 cm, and 20%, respectively. The majority of the cases were IDC, with a median Ki-67 index of 22%.[16]

Nishimura et al recognized Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer.[17]

A higher Ki-67 index (>20%) was significantly correlated with a higher tumor grade in the Japanese study.[18] A study revealed Ki -67, a marker of cellular proliferation in breast cancer, was and can be used in addition to other clinical and/or pathological features to identify patients whose cancer may be at higher risk of recurrence.[19]

A study includes breast cancer patients from a single institute to evaluate the interaction between Ki-67 expression levels and histological grade and their prognostic role in different IHC-based breast cancer subtypes.

The histological grade is an important determinant of breast cancer prognostication and could be incorporated into staging systems and algorithms to choose the most appropriate treatment for patients with breast cancer. Therefore, the tumoral grade may be indirectly related to Ki-67 expression based on the mitotic count.[20, 21]

Last but not the least direct correlation of Ki67 positivity with high histological grade in the current study stresses the need for Ki67 as a valuable marker for the diagnosis of breast carcinoma. In this regard, we recommend that immunostaining of Ki67 should be used routinely for breast cancer diagnosis as It will help in the early and timely management of high-risk patients to reduce the morbidity and mortality associated with breast carcinoma.

CONCLUSION

Ki67 expression in breast carcinoma is usually seen in rapidly dividing cells in aggressive tumors and the present study expression of Ki67 was observed in advanced tumors.

There is a direct correlation between Ki67 positivity with a high histological grade as High expression of Ki67 correlates with a higher histological grade. Also, Ki67 has an important role in the selection of appropriate therapy for breast carcinoma; we recommend immunostaining of Ki67 should be used routinely for diagnostic evaluation of breast cancer as it will help in the early and timely management of high-risk patients to reduce the morbidity and mortality associated with breast carcinoma

LIMITATIONS

The main limitations of this study were that the sample size is small and a larger sample size can provide further avenues for determining histopathological correlation.

STRENGTHS

The main strength of this study is the fact that it establishes a direct histological correlation between the increased expression of Ki67 and different histological grades of invasive ductal carcinoma breast.

This significance can be employed for assessing the prognosis as well as determining the need for adjuvant chemotherapy in the patients.

CONFLICT OF INTEREST / DISCLOSURE

The authors declared no conflict of interest.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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