Immunohistochemical expression of Ki-67 in invasive breast carcinoma and its correlation with prognostic parameters

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Abstract

Background: Ki-67 expression is strictly associated with cellular proliferation and is an excellent marker for determining the growth fraction of a given cell population. It may be considered as alternative to mitotic counts in grading schemes and as a single parameter that can be used in fine needle aspirates and small biopsies. The aim of this study was to evaluate proliferative fraction of Ki67 expression in invasive breast carcinoma and to find out the correlation between Ki67 expression and clinico-pathological prognostic factors such as tumor size, lymph node status, histological grade and Nottingham prognostic index. **Materials and Methods:** Seventy cases of infiltrating ductal carcinoma breast were studied. Immunohistochemistry using the anti-Ki-67 antibody [MIB-1] was performed on all cases and Ki-67 staining was categorized as low and high grade. **Result:** High expression of Ki-67 was found in 71.05% cases of breast tumor with positive lymph node metastasis and 92.31% cases of Grade III tumor. Ki-67 expression was significantly associated with nodal status (p<0.0001) and poorly differentiated tumor (p<0.0001). Although, Ki-67 expression was associated with tumor size, but their correlation was not statistically significant (p= 0.065). **Conclusion:** High Ki-67 expression was associated with a higher grade of malignancy. Conclusively, proliferative activity determined by Ki-67 expression may reflect the aggressive behavior of breast carcinoma. Ki-67 detection provides valuable information about proliferative activity of cells so, it is necessary to combine this with other parameters for better prognosis.

Keywords: Breast carcinoma, Histological grade, Ki-67

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Introduction

Carcinoma of breast is the most common malignant tumor in women. Evaluation of breast lumps is essential to identify patients with benign proliferative lesions of the breast and these benign lesions are an important risk factor for subsequent transformation to malignancy [1]. Advances in imaging techniques and the increased use of needle biopsy have greatly assisted the preoperative evaluation of breast lesions but, in a large proportion of cases, differentiation between benign and malignant lesions still rests on histological examination [2]. Prognostic factors provide information which is useful in assessing the outcome at the time of diagnosis.

Traditional prognostic factors in patient with invasive breast cancer include lymph node status (the single most important prognostic factor), tumor size, histological type and histological grade [2]. In addition to the traditional histopathological parameters, the assessment of proliferation is the most important factor for treatment decisions in breast cancer patients [3]. A wide range of techniques are available to assess tumor cell proliferation such as percentage of cells in mitosis, thymidine or Bromodeoxyuridine (BRDU) labeling index, S phase fraction measurement, examination of proliferating cell nuclear antigen (PCNA) and immunostaining with antibodies to Ki-67 [2,4,5]. Ki-67 antigen is a huge nonhistone nuclear protein of approximate 395 kDa, encoded for almost 30,000 base

pairs within the human genome [6]. Ki-67 protein was originally defined by the prototype monoclonal antibody Ki-67. Ki-67 monoclonal antibody was generated by immunizing mice with nuclei of the Hodgkin's lymphoma cell line L428 [7]. Ki-67 nuclear antigen is being associated with cellular proliferation. Except G0 phase, it is expressed in S, G1, G2, and M phases of the cell cycle [8]. The most prevalent analysis method of Ki-67 antigen is the immunohistochemical evaluation [9]. Ki-67 values are defined as the percentage of positively marking malignant cells using the antihuman Ki-67 monoclonal antibody MIB-1 which is the most commonly used antibodies and considered as the "gold standard" [10]. Growth fraction of neoplastic cell populations is assessed by immunostaining with Ki-67monoclonal antibody. Ki-67 detection provides significant evidence of the proliferative fraction for chemotherapy decision in the early breast cancer and is used to predict the risk of recurrence and extent of chemotherapy benefits in women with node-negative ER-positive breast cancer [11, 12]. Many studies have assessed the association between Ki-67 immunoreactivity with other prognostic factors in breast carcinomas [13, 14].

The objective of this study is to evaluate proliferative fraction of Ki67 expression in invasive breast carcinoma and to find out the correlation between Ki67 expression and prognostic markers such as tumor size, lymph node status, histological grade (Modified Bloom Richardson Grade) and Nottingham prognostic index (NPI).

Materials and Methods

This was a retrospective study carried out in the Department of Pathology, Gandhi Medical College, Bhopal (M.P.). Seventy cases of invasive ductal breast carcinoma, not otherwise specified (IDC, NOS) after mastectomy were studied, after permission from institutional ethics committee. All cases of male breast carcinoma, carcinomas other than invasive ductal carcinoma (NOS), breast carcinoma in pregnant woman and cases exposed to radiotherapy or chemotherapy were excluded from this study. Patient's demographic data such as age, details of tumor size and lymph node status were collected from archives of our department. Paraffin blocks of the selected cases were retrieved for immunohistochemical (IHC) staining of Ki-67.

Paraffin blocks of all selected cases were processed and stained by haematoxylin and eosin staining; and reviewed for histological type and grade. Grading was done according to Modified Bloom Richardson Grade [2]. Size of the tumor was classified as pT1 ($\leq$2cm), pT2 (>2cm to $\geq$5cm) and pT3 (>5cm); and lymph node status was classified as pN0, pN1 (1-3), pN2 (4-9) and pN3 (>10) according to the TNM staging system. Nottingham prognostic index (NPI) was calculated in all cases as follows-

Tumor size in cm x 0.2 + lymph-node stage (I, II or III) + histologic grade (1, 2 or 3) and categorized in the following groups: A. < 3.4 (Good prognosis), B. $\geq$ 3.4 and <5.4 (Moderate prognosis), C. $\geq$ 5.4 (Poor prognosis).

All sections were screened and representative blocks were selected for immunohistochemical (IHC) staining. We used Biogenix USA kit for immunohistochemical (IHC) staining. 3 to 4 µm thin sections were taken on slides coated with 1% poly lysine O. Sections were deparaffinised in xylene and rehydrated in absolute alcohol, 90% alcohol and 70% alcohol. Antigen retrieval was done in pressure cooker using tri sodium citrate buffer at pH 6. Sections were treated with 3% hydrogen peroxidase to quench endogenous peroxidase activity and then stained with antibodies against Ki-67 for 2 h (positive and negative controls were run simultaneously). The peroxidase antiperoxidase method was followed for secondary staining. DAB [3, 3'- Diaminobenzidine] was used for coloring the antigen–antibody complex.

The proportion of the malignant cells staining positive for the nuclear antigen Ki-67 was evaluated in a quantitative and visual way by using light microscope. IHC slides were initially screened under 40 magnifications, to identify areas of maximum intensity. The Ki-67 score is defined as the percentage of positively stained tumor cells among the total number of malignant cells assessed [15].

Distinct nuclear staining was taken as positive. Germinal centre of reactive lymph node was taken as positive control. A minimum five hundred cells were counted in an area of maximum Ki67 positivity and expressed as percentage. A Ki-67 cut-off point of 15% was defined according to the experience of different pathologists [4, 14, 16]. A count less than 15% was taken as low grade [Fig-1] and more than 15% was taken as high grade [Fig-2].
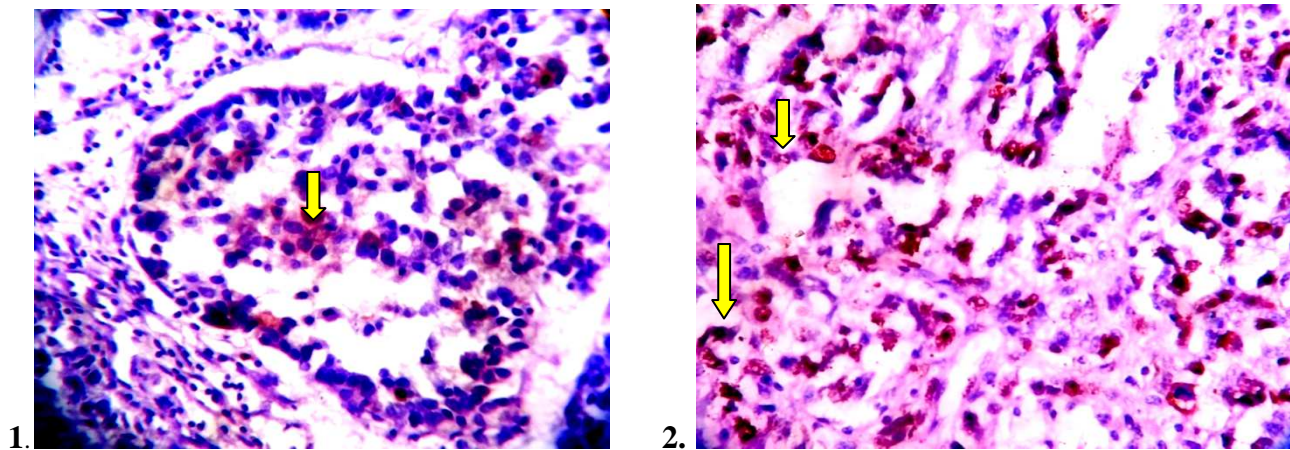
Figure 1& 2: Immunohistochemical (IHC) staining of low Ki-67 expression (<15% nuclear staining) and high Ki-67 expression (>15% nuclear staining) in figure 1 and 2 respectively. Arrow shows cells with distinct nuclear staining for Ki-67. [400x]

Statistical analysis of data was performed by using Statistical Package for Social Sciences (SPSS) version 15.0 (Chicago, IL). Categorical data were expressed as frequency counts (percentages)**.** Statistical correlation for categorical data was done by using Chi-square test. A *P* value < 0.05 was considered significant.

Results

The associations between Ki-67 expression and clinico-histopathological parameters are shown in Table-1. The incidence of invasive breast carcinoma was highest (34.28%) in the age group of 41-50 years. Expression of Ki-67 (>15%) was

Table 1: The association between the Ki-67 expression and clinico-histopathological parameters in breast cancer (n=70)

Clinico-histopathological parameters		Ki-67 expression		P value*
		Low grade (<15%)	**High grade (>15%)**	
Menstrual history	Premenopausal	11 (39.28%)	17 (60.72%)	0.039[a]
	Postmenopausal	27 (64.29%)	15 (35.71%)	
Tumor size	T1	3 (100%)	0	0.065[b]
	T2	18 (64.28%)	10 (35.72%)	
	T3	17 (43.58%)	22 (56.42%)	
Lymph node	Negative lymph node (pN0)	27 (84.37%)	5 (15.63%)	< 0.0001[a]
	pN1	7 (41.17%)	10 (58.83%)	
	pN2	4 (22.22%)	14 (77.78%)	
	pN3	0	3 (100%)	
	Positive lymph node [#] (pN)	11 (28.95%)	27 (71.05%)	
Tumor grade	Grade I	19 (100%)	0	< 0.0001[a]
	Grade II	18 (47.36%)	20 (52.64%)	
	Grade III	1 (7.69%)	12 (92.31%)	
NPI score	< 3.5	17 (100%)	0	< 0.0001[a]
	> 3.5 to < 5.5	16 (61.54%)	10 (38.46%)	
	>5.5	5 (18.52%)	22 (81.48%)	

*Statistical analysis of data was done by using Chi-square test. *P* value < 0.05 was considered significant. a. Significant, b. Not significant. # Positive lymph node (pN) is the sum of pN1, pN2 and pN3.

high in 32 (45.72%) cases. High expression of Ki-67 was found in 60.72% and 35.71% cases of premenopausal and postmenopausal women respectively. The correlation between menstrual history and Ki-67 expression was statistically significant [p=0.039]. Regarding the tumor size, we observed that low pT stage tumors were associated with low expression of Ki-67, whereas high pT stage tumors were associated with high expression of Ki-67. But the association between Ki-67 expression and tumor size was not statistically significant [p=0.065]. Concerning the nodal status, high expression of Ki-67 was found in 71.05% and 15.63% cases of breast tumor with positive lymph node and negative lymph node respectively. We observed that as the nodal status of the tumor increases, expression of Ki-67 was also increases. The association between Ki-67 expression and nodal status of tumor was statistically highly significant [p<0.0001]. We found that in Grade I tumor, all cases were associated with low Ki-67 expression, whereas in Grade III tumor, 92.31% cases were associated with high Ki-67 expression. High Ki-67 expression was significantly [p<0.0001] associated with advanced stages and poorly differentiated tumor. We also found that expression of Ki-67 increases, as the NPI value increases, which means high Ki-67 expression, was associated with poor prognosis.

Discussion

Numerous studies have shown that morphological assessment of tumor differentiation provides prognostic information in the patients with breast carcinoma [17, 18]. At present, prognosis of breast carcinoma is predicted on the basis of clinicopathological parameters such as tumor size, nodal status, histological type and nuclear grade. The rate at which a tumor proliferates has been correlated with its clinical course. Histopathologists, therefore, have sought a means of determining the rate of tumor proliferation as an adjunct to diagnosis. The simplest and most established of these practices is a count of mitotic figures. The use of antibodies to Ki-67 is a reliable and easy means of accurately assessing the growth fraction of human neoplasms. A perfect correlation has been demonstrated between visual counting techniques and image analyzing systems for calculating the percentage of cells stained with Ki-67 [19]. Many authors have reported an association of Ki-67 with histological grade, lymph node status, tumor size, ER/PR status and p53status [20, 21, 22]. Ermiah E. et al found that high Ki-67 expression was associated with a higher grade of malignancy such as hormone receptor negative tumors, advanced pT stages, poor differentiation of tumors, positive axillary lymph nodes, distant metastasis and a high S -phase fraction [23]. Jacquemier et al observed that vascular and lymphatic invasion were associated with high Ki-67 expression [24]. Inwald E.C. et al also observed that high Ki-67-labeling index was associated with larger tumors, higher tumor grade, vascular invasion, and HER-2 positivity [16]. In the present study, we found that low pT stage tumors were associated with low expression of Ki-67, whereas high pT stage tumors were associated with high Ki-67 expression. We also observed that higher nodal status were significantly associated with higher Ki-67 expressions. These findings are similar to the findings reported by earlier researchers [16, 23]. Histological

grading is commonly used parameter for therapy decision. Grading describes differentiation as well as proliferation in various tumors. We found that higher Ki-67 index (>15%) was significantly correlated with a higher grade of malignancy. This correlation was proven in various former studies [16, 22, 23]. Ki-67 expression is used as a prognostic factor for both disease free survival (DFS) and overall survival (OS), irrespective of the lymph node status [25]. Inwald E.C. et al found that patients with high Ki-67 labeling index breast tumor had both worse disease free survival (DFS) and overall survival (OS) [16]. Midulla et al, observed that high level of Ki-67 expression was associated with early recurrence after mastectomy and low survival rate [26]. We found that expression of Ki-67 increases, as the NPI value increases, which means high Ki-67 expression, was associated with poor prognosis. Most of the studies were outlining the importance of Ki-67 to predict the clinical and pathological response to chemotherapy in early or locally advanced breast cancer. There is a tendency of reduction of Ki-67 value after chemotherapy [27]. Dowsett et al, observed that short term endocrine treatment may improve the disease free survival which can be predicted by measurements of Ki-67 level. This finding suggests that the Ki-67 is an important marker throughout the course of treatment [28].

Conclusion

This study confirms the value of Ki-67 evaluation as a prognostic factor being associated with breast cancer outcomes. High Ki-67 expression was associated with a higher grade of malignancy such as advanced pT stages, positive axillary lymph nodes and poor differentiation of tumors. Conclusively, proliferative activity determined by Ki-67 expression may reflect the aggressive behavior of breast carcinoma. Ki67 is a very

reliable replacement for mitotic count and can be determined easily by using immunohistochemistry in tumor samples obtained by needle core biopsies and in cytologic specimens, in which there are limited number of cells present. Neoadjuvant therapy, including preoperative chemotherapy and hormonal therapy, is becoming increasingly for early breast cancer. It is desirable to grade tumors using Ki-67 labeling index before surgery so that the most appropriate medical regimen can be selected.

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