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Ki-67 Expression in Primary Breast Carcinomas and Their Correlation Nottingham Modification of Scarff Bloom Richardson Grading System

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Abstract

Background: Carcinoma breast is the one of the most common causes of cancer death among women. Early diagnosis and effective treatment of the disease are immensely important. The increasing number of options for treatment of carcinoma breast has made the prognostic evaluation of the disease even more important. Proliferation plays an important role in clinical behaviour of invasive carcinoma breast. Ki-67 binding, is an objective measurement of cell proliferation which significantly aids in the management of the patients with carcinoma breast. *Objectives:* 1. Study of expression of Ki-67 in primary breast carcinomas. 2. Correlation of Ki-67 expression with the histopathological grade of breast carcinomas. *Methodology:* Forty modified radical mastectomy specimens received at the department of pathology, MIMS, Mandya from January 2015 to June 2016 were examined for gross and microscopic features. Immunohistochemistry was used to study the Ki-67 expression. Ki-67 status was correlated with tumour grade according to Nottingham modification of Scarff Bloom Richardson grading (NSBR grading. *Results:* Of 40 cases of carcinoma breast, 97.5% of the cases were positive for Ki-67. The range of Ki-67 score was 0-95%. Mean Ki-67 in the study was 32.85%. Majority of the cases (62.5%) had high Ki-67 expression. There was a significant statistical association between Ki-67 expression with grade of the tumor(p value = 0.032). *Conclusion*: Our study data indicates that, high Ki-67 expression is associated with high tumour grade. Conclusively, proliferative activity determined by Ki-67 expression may reflect the aggressive behaviour of breast carcinoma. Ki-67 detection provides valuable information about proliferative activity of cells therefore, it is necessary to combine this with other prognostic parameters for better evaluation of patient's outcome as well as treatment decision.

Keywords: Immunohistochemistry; Ki-67; Breast Carcinoma; Nottingham Modification of Scarff Bloom Richardson Grading (NSBR Grading).

Introduction

Breast carcinoma is the most common malignant tumour and the leading cause of cancer death in women, with more than one million cases occurring worldwide annually. The incidence curve starts rising at puberty, increases steeply up to menopausal age, and levels off afterwards [1].

Prognostic information is important in counselling patients about the likely outcome of their disease and planning further management. Apart from clinical parameters like age, menopausal status and disease presentation, important prognostic indicators in histopathology are tumour size and extent, histological type, histological grade, lymph node status. In addition, there are other factors like, hormone receptor status and HER-2/neu status which not only are predictive of outcome, but also direct therapies against particular molecular targets [2].

Uncontrolled proliferation is the hallmark of malignancy and it plays a very important role in clinical behaviour of invasive breast carcinoma. Increased proliferation correlates strongly with poor prognosis. There are various methods for assessing the proliferation like counting of mitotic figures on stained section, incorporation of labelled nucleotides into DNA and flow cytometric evaluation of the fraction of the cells in S-phase [3]. Of all, counting of mitotic figures on stained section has been shown to provide reproducible and independent prognostic

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value. However, Ki-67 antigen assessment by immunohistochemical method is a promising objective alternative [4].

The choice between hormonal therapy which has minimal side effects and chemotherapy with well-known morbidity and risks is a major concern of the clinician. Response to hormone and chemotherapy depends on the proliferating activity of the tumour. Patients with tumours having high proliferating activity responds well to chemotherapy compared to hormonal therapy [5].

Hence the present study is being undertaken to establish a correlation between the proliferative activity and tumour histopathology to further provide additional information regarding prognosis for therapeutic implication.

Methodology

This study was done including forty modified radical mastectomy specimens of carcinoma breast submitted to the Department of Pathology, MIMS, Mandya for histopathological study during the period from January 2015 to June 2016.Clinical data were obtained from patient's clinical history and examination, hospital records and requisition forms received in the Department. The specimens were examined for gross and microscopic features. The tumours were typed according to the WHO classification system [6]. The Nottingham modification of Bloom Richardson grading system was used for grading.

All the 40 cases were subjected to IHC study for Ki-67 from the representative areas of the tumour.

- Ki-67 immunoreactivity was recorded as continuous variables, based on the proportion of positive tumor cells (0-100%) regardless of staining intensity.
- Besides evaluating Ki-67 as continuous variable, levels of Ki-67 were quantified as low and high by taking Ki-67 cut-off as 15%.

Then the Ki-67 score was correlated with grade of tumour.

Plan of Data Analysis

The collected data was entered in Excel sheet and analyzed using SPSS software and the descriptive statistics, one-way ANOVA test and Chi-square test were applied for the data as applicable. The p value of <0.05 was considered statistically significant.

Results

In the present prospective study, a total number of 40 female patients with carcinoma breast were evaluated. The age of patients with carcinoma breast ranged from 27 to 86 years. All the 40 cases presented with lump in the breast, 15 cases with pain in addition to lump and 05 cases with ulceration of overlying skin in addition to the breast lump (Fig. 1).

Of the 40 cases, 23 (57.5%) cases had tumour in the left breast and 16 (40%) in the right breast. One case (2.5%) presented with bilateral involvement of the breast. The tumour was in the upper outer quadrant (UOQ) in 26 (65%) cases, upper inner quadrant in 02 (5%) cases and central in location in 03 (7.5%) cases. In 9 (22.5%) cases the tumour was involving multiple quadrants.

The largest dimension of tumour ranged from 2 cm to11 cms. Majority of the cases (38 cases, 95%) were of infiltrating ductal carcinoma, NST type (Fig. 2), followed by one case each of infiltrating lobular carcinoma, and mucinous carcinoma.

The histopathological grade was studied and recorded according to NSBR system. Of the 40 cases of carcinoma breast studied, majority of the cases (18 of 40; 45%) were of grade 2 followed by 12 (30%) cases of grade 3 and 10 (25%) cases of grade 1.

All cases in the present study were subjected to Ki-67 staining by IHC method. 39 (97.5%) of the 40 cases were positive for Ki-67 and one was negative. The range of Ki-67 score was 0-95%. Mean Ki-67 in the study was 32.85%.

The mean value of Ki-67 in IDC-NST type was 32.73%. There was one case of lobular and mucinous carcinoma each, which showed Ki-67 score of 10% and 60% respectively.

Ki-67 cut off point of 15% was used to define the breast cancer as tumour with low proliferation (d"15%) and high proliferation (>15%). Majority of the cases (25 of 40, 62.5%) in the present study had high proliferation (Fig. 3) and the rest (37.5%) had low proliferation (Fig. 4).

The mean Ki-67 score was studied in different grades of tumour. The mean Ki-67 in grade 1, grade 2 and grade 3 tumours was 24.3%, 33.78% and 38.5% respectively. Although there was in increase in the mean Ki-67 score as the tumour grade worsens, there was no statistically significant association between the mean Ki-67 score and histopathological grade when Ki-67 was taken as a continuous variable with p value of 0.503 (Table 1).

Table 1: Table showing correlation of grade of tumour and mean Ki-67

Grade	No. of Cases	Mean Ki-67(%)	p value
Grade 1	10	24.30	0.503
Grade 2	18	33.78	
Grade 3	12	38.50	
Total	40	32.85	

Table 2: Table showing correlation of grade of tumour with groups of Ki-67

Grade	Low Ki-67 index (N= 15)	High Ki-67 index (N=25)	Total (N=40)	p value
Grade 1	7(70.0%)	03(30.0%)	10(100%)	**0.032**
Grade 2	6(33.3%)	12(66.7%)	18(100%)	
Grade 3	2(16.7%)	10(83.3%)	12(100%)	

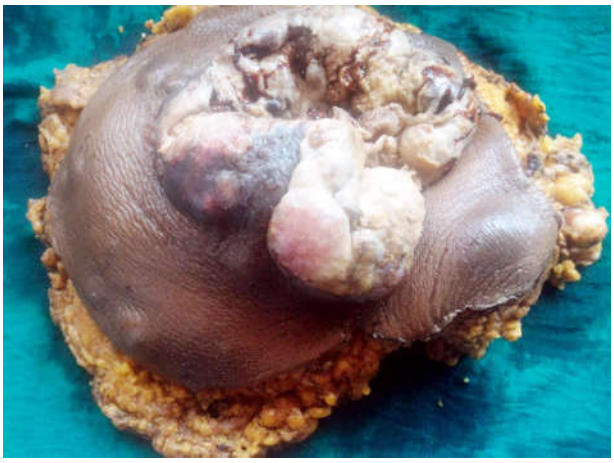

Fig. 1: Mastectomy specimen – Showing ulceration of overlying skin and destruction of nipple and areola

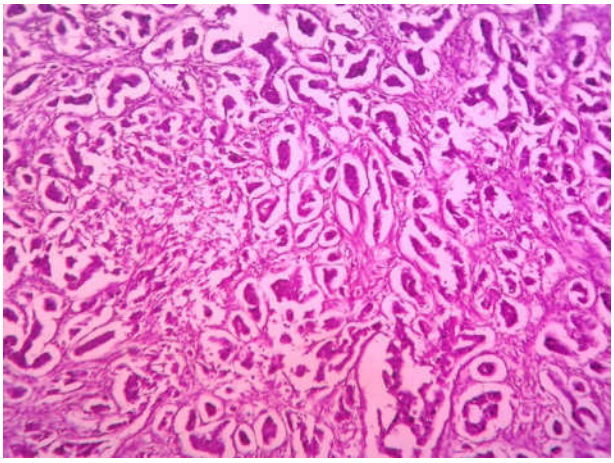

Fig. 2: Microphotograph of grade 1 Infiltrating duct carcinoma, N)ST type (H & E, X100)

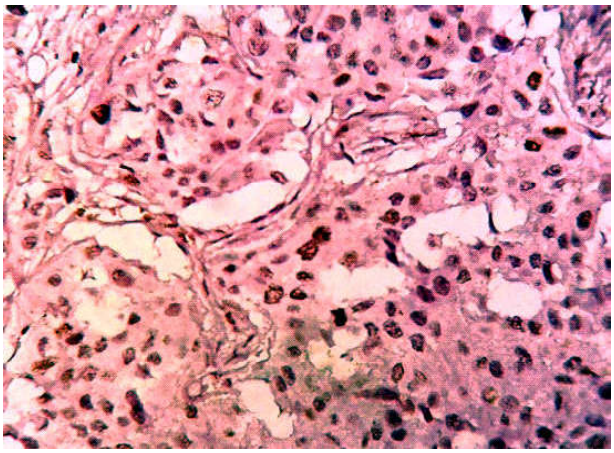

Fig. 3: Microphotograph showing high Ki-67 expression (DAB, X100)

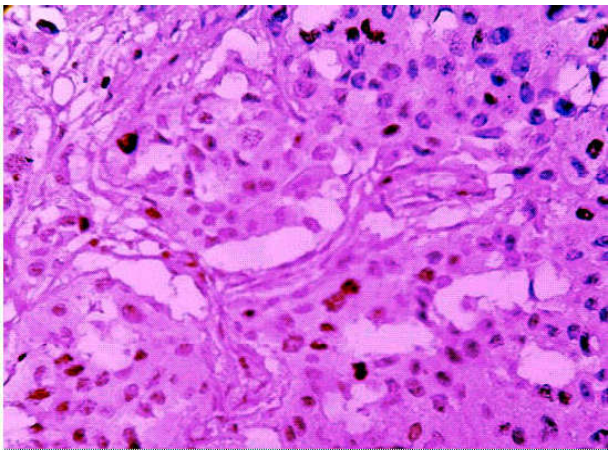

Fig. 4: Microphotograph showing low Ki-67 expression (DAB, X100)

Further the grade of the tumour was correlated with the groups of Ki-67 positivity. Most of the tumours in the grade 3 (10 of 12; 83.3%) had high Ki-67 proliferation index as compared to grade 1 tumours, wherein 3 of 10 cases (30%) had high proliferation index. This relation was statistically significant with p value of 0.032 (Table 2).

Discussion

Biomarker expression in breast cancer is used as a prognostic indicator and predictor of response to chemotherapy. To date, the leading parameters that guide adjuvant therapy in breast cancer are ER, PR and HER 2/neu along with IHC for Ki-67 to determine tumour proliferation [87].

Several studies have investigated the prognostic significance of Ki-67 in breast cancer. The present study was done to evaluate the prognostic significance of Ki-67 in breast cancer by correlating with tumour grade, a traditional well known prognostic marker.

In our study, the age group ranged from 27-86 years with the mean age of 49.45 years which was similar to studies by Nishimura et al [7], Inwald et al [8] and Haroon et al [9]. Majority of the cases (70%) in our study had the tumour size ranging from 2-5 cms, followed by 30% cases of tumour size of > 5 cms. This finding is similar to the study of Haroon et al [9] and in contrast to other studies. Ermiahet al [10] did a study in Libya where patients present at a later stage of disease, thus in their study 64% of cases had tumour size of > 5cm. However, in studies done by Wojnaret al [11] and Inwald et al [8], majority of the cases had tumour size of < 2cm.

In the present study, majority of the cases (18 of 40; 45%) were of grade 2, followed by grade 3 (12 of 40; 30%) and grade 1 (10 of 40; 25%). Similar findings were noted in the studies by Bottiniet al [12], Inwald et al [8], Ermiah et al [10], Wojnar et al [11] and Haroon et al [9].

Ki-67 was positive in 39 (97.5%) of the 40 cases. The range of Ki-67 score was 0-95%. Mean Ki-67 in the study was 32.85%. These values were comparable with other studies by Mcgurrinet al [13], Bouzubar et al [14], Molino et al [15], Bankfalvi et al [16] and Haroon et al [9].

The cut-off point according to St Gallen's consensus is 14% to differentiate luminal A and Luminal B tumours. However, various studies use different cut-off values. For this reason we had limited numbers of inter study comparability. Hence, In the present study we consider the cut-off point as 15% to designate the tumour into low and high proliferation based on the experience of different pathologists as well as national and international recommendations at present [8].

In the present study, majority of cases (25 of 40; 62.5%) had high Ki-67 expression and only 37.5% (15 of 40) had low Ki-67 expression which was similar to other studies done by Haroon et al [9] and Ingolf et al [17]. This was in contrast to study done by Jain et al [18] where majority of patients had low Ki-67 expression. This difference is due to their study consisting predominantly of low-grade, node-negative tumours.

In present study, 63.2% of cases had high Ki-67 index and 36.8% of cases had low Ki-67 index which was similar to a study by Thanget al [19] which also had majority of cases which was expressing high Ki-67. In contrast, a study by Srebnijset al [20] had no such difference.

Majority of cases 18 (45%) in the study were of grade 2 followed by grade 3 and grade 1 (30% and 25% respectively). The mean Ki-67 in grade1, grade 2 and grade 3 tumours was 24.3%, 33.8% and 38.5% respectively. Although there was in increase in the mean Ki-67 score as the tumour grade increases, results were not statistically significant. A positive association between the tumour grade and mean Ki-67 was found in studies by Haroon et al [9] and Inwald et al [8].

However, when the grade of the tumour was correlated with the groups of Ki-67 score, we found that only 30% of grade 1 tumour had high proliferation whereas the grade 2 and grade 3 tumours had high proliferation of 66.7% and 83.3% respectively. These results were statistically significant which was similar to other studies by Haroon et al [9] and Jain et al [18].

Conclusion

Carcinoma breast is a heterogeneous disease. In addition to conventional histopathological parameters, the assessment of proliferation of is one of the important factors for the treatment decisions of the patients with carcinoma breast. Proliferative activity determined by Ki-67 may reflect the aggressive behaviour of the carcinoma breast and aids in the treatment decision.

Our study evaluated Ki-67 positive immunoreactivity and correlated with one of the established prognostic factor, NSBR grade in 40 cases of carcinoma breast. According to the data of our study, a significant statistical association was found between Ki-67 proliferation index and grade of tumour.

In conclusion, our study indicates that Ki-67 may be a more powerful predictor for poor prognosis as its expression is associated withhigh tumour grade. Ki-67 immunostaining can be considered as a convenient method for assessing the proliferative index, since, unlike other markers, it is a rapid and cost effective technique that can be easily adopted in almost all Pathology laboratories.

In future it is necessary to carry out studies with large samples of carcinoma breast and using other molecular prognostic markers to evaluate the prognosis and to provide better therapeutic options.

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