

A Study to Correlate Ki-67 Proliferative Activity in Lymph Node Positive and Lymph Node Negative Cases of Breast Carcinoma

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Abstract: Breast cancer is the most common cancer in women worldwide. In this study 43 patients were included into this part prospective and part retrospective study. Ki 67 assessment was done on tumor tissue and lymph node involvement was determined by histopathological analysis. The association between lymph node positivity and Ki67 was analyzed. Lymph node involvement was found in 24 patients. Mean Ki-67 score for lymph node negative and positive cases of breast carcinoma was 11.89 and 17.54 respectively. T-test applied and the relation between them found is statistically significant with p- value 0.044. The high levels of Ki-67 were positively correlated with a higher incidence of lymph node metastasis. Our data suggest that Ki-67 positivity has value as a prognostic and predictive biomarker in breast cancer and may be a valuable proliferation marker in routine diagnosis of breast cancer

Keywords: Ki 67, Axillary nodes, Immunostaining.

INTRODUCTION

Breast cancer is the second most common type of cancer worldwide and by far the most common cancer among women [1]. Incidence of breast cancer is rapidly rising in India and is estimated to overtake the cervical cancer soon [2]. Proliferation is a key feature of progression of tumours and is widely estimated by the immunohistochemical assessment of the nuclear antigen Ki-67. Ki-67 was identified by Gerdes *et al.* as a nuclear non histone protein [3]. The Ki-67 gene is on the long arm of chromosome 10(10q25) [6].

Ki-67 expression is usually estimated as the percentage of tumour cells positively stained by the antibody [4]. Over the years there have been quite a few studies which have tried to correlate Ki-67 immunostaining with other indicators of prognosis and have also found it to be an independent prognostic marker in these tumors [5]. In their study, Bouzubar *et al.* found that a level of Ki-67 immunostaining was often associated with early recurrence of breast cancer after mastectomy [6]. Lymph node status is said to be a strong clinical indicator of prognosis in carcinoma breast. But the correlation of Ki-67 immunostaining with this strong independent prognostic maker is variable. A study by Wrba and Wintzer showed a positive correlation between these two parameters [7,8]. Bouzubar and Barnard in their respective studies found lymph node status to be unrelated to Ki-67 positivity. As regards, the relation of Ki-67 positivity to metastasis, the results are variable. Bouzubar *et al.* found no significant correlation of these two whereas in the study of Mc Gurrin *et al.* though, no significant relation could be derived, 90% of patients with more than 30% Ki-67 positivity had distant metastases [9].

Though it is reasonably well established that Ki-67 positivity is an independent predictor of ultimate prognosis and course of carcinoma breast and its recurrence, many lacunae exist. The study is aimed to correlate Ki-67 Labelling Index with lymph node status of breast carcinoma in Indian population.

MATERIALS AND METHODS

The study was carried out in Department of pathology, Vardhman Mahavir Medical Collage and Safdarjung Hospital, New Delhi. A part retrospective and part prospective study was done on a total of 43 cases of carcinoma breast.

Case selection

43 representative paraffin blocks of Modified Radical Mastectomy of carcinoma breast were included for the study.

Inclusion criteria

- MRM specimens with nodal tissue within it.

Exclusion criteria

- Tissue blocks with extensive necrosis or hemorrhage.
- Tissue section with inadequate study material.

4-5 micron thick sections were cut for Hematoxylin and Eosin (harris method) staining.

All stained slides were reviewed and the morphological diagnosis based on H & E staining was considered as a gold standard to be compared with Ki-67 staining.

For Ki-67 immunohistochemistry, representative sections from paraffin blocks were taken on poly- L- lysine coated glass slides. Positive and negative controls were run with each batch of IHC stain. Positive controls included sections from invasive breast cancer known to express Ki-67. Negative

control staining was obtained by substituting primary antibody with an antibody of irrelevant specificity.

Ki -67 score: It is the percentage of tumor cells showing the nuclear positivity for Ki-67. For counting cells positive for Ki-67 immunostaining we used random counting method. Thus, percentage of tumor cells in 10 randomly chosen fields from most representative invasive areas with minimum 1000 cells were counted.

RESULTS

In present study, maximum number of cases were in age group of 41-50 years constituting 44.18% of all cases while cases were minimum in the age group of 11- 20 year (2.32%). Mean age of presentation was 43.74 years.

Out of total cases, 93% were females while males constituted only 7% of all cases.

Table-1: Age distribution

Age Group (Yrs)	No. of Cases
11-20	1 (2.32%)
21-30	3 (6.97%)
31-40	10 (23.25%)
41-50	19 (44.18%)
51-60	5 (11.63%)
61-70	5 (11.63%)
Grand Total	43 (100.0%)

Table-2: Sex distribution

Sex	No. of Cases
Female	40 (93%)
Male	3 (6.97%)
Grand Total	43 (100%)

Table-3: lymph node status

Lymph node status	Number of cases	%
Negative	19	44.19
Positive	24	55.86
Total	43	100

Table-4: Age and lymph nodes

Age group (year)	Lymph node negative	Lymph node positive
11-20	1	0
21-30	2	1
31-40	5	5
41-50	7	12
51-60	2	3
61-70	2	3
Grand total	19	24

Table 4 shows that both lymph node negative and lymph node positive cases were maximum in age

group of 41 -50 years. Only single case of negative lymph node was reported in 11-20 years age group

Table-5: Correlation between ki-67 and lymph nodes

Lymph node	Mean Ki-67 score	Std. deviation
Negative	11.89	10.52
Positive	17.54	10.42

PROBABILITY VALUE – LESS THAN 0.044

In table 5, mean Ki-67 score for lymph node negative and positive cases of breast carcinoma was

11.89 and 17.54 respectively. T-test applied and the relation between tem found be statistically significant with p- value 0.044.

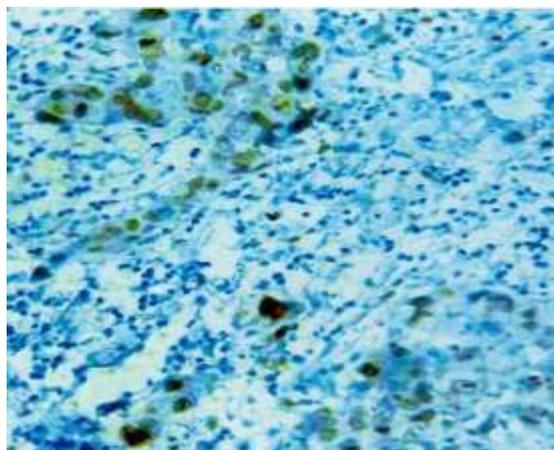


Fig-1: Ki-67 Immunostaining in Invasive Ductal Carcinoma Grade III.(IHC; ×400)

DISCUSSION

In present study, lymph node positive cases constituted 24 while lymph node negative cases were 19. Most lymph node positive cases were seen in the age group of 41-50 year. This group also contained maximum lymph node negative cases. In their study Hernandez *et al.* found that the 5 year overall survival was 80% for node- negative patients (N 0), 65% for one to three positive lymph nodes(N 1), 48% for four to nine positive lymph nodes (N2), and 44% for ≥10 positive lymph nodes[10]. Ki-67 immunostaining was also correlated with lymph node status of the cases. The mean value of Ki-67 in lymph node negative and lymph node positive cases were 11.89 and 17.54 respectively. The p-value was calculated and came out to be 0.044 showing statistically significant relation. Lymph node status is said to be a strong clinical indicator of prognosis in carcinoma breast. But the correlation of Ki-67 immunostaining with this strong independent prognostic marker is variable. Wrba *et al.* and wintzer *et al* showed a positive correlation between these two paraments [7,8]. Bouzubar and Barnard in their respective studies found lymph node status to be unrelated to Ki-67 positively[9]. Zhaohui *et al.* demonstrated that the high Ki67 LI in primary tumors correlates with increased risk of axillary lymph node metastasis in triple-negative medullary breast carcinoma patients, predicting that Ki67 provides prognostic information in triple-negative medullary breast carcinoma patients[11]. The high levels of Ki-67 positive (greater than 20 %) were positively correlated with a higher incidence of lymph node metastasis by Yongxiang *et al* in a study of 147 patients [12].

CONCLUSION

On the basis of observations of this study, it may be concluded that Ki-67 can be used along with lymph node status to assess the proliferative potential of the cancers of breast and hence may be considered as a significant factor of breast cancer. Further studies may be required on large samples to assess the Ki-67 role in breast cancer and to consider it as a significant prognostic factor of breast cancer.

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