Estimation Of Ki-67 Positivity In Relation To Size Of Tumor And Age Of Patient In Invasive Breast Carcinoma

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ABSTRACT

Background: Identification of cells in the replicative pool for cases of breast cancers and their association with various conventional prognostic parameters would have widespread predictive as well as therapeutic value. Cell proliferation studies would supplement existing markers for devising appropriate treatment protocol for individual cancer cases.

Objective: To evaluate proliferative activity via Ki-67 immunohistochemical labeling in invasive breast carcinoma and to find its relationship to age of the patient and size of tumor at the time of diagnosis.

Materials & methods: This Descriptive Study was carried out at Department of Pathology, Basic Medical Sciences Institute, JPMC, Karachi, Pakistan, Formalin-fixed-paraffin-embedded tissues of 50 cases of diagnosed invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC) with and without lymph node involvement were retrieved. Immunohistochemical staining for Ki-67 antigen was performed. The number of positively stained nuclei in 1000 tumor cells in at least five representative high power fields was counted. "EPI-INFOR" was used for statistical analysis.

Results: 33 cases showed positive nuclear staining for Ki-67 antigen with 08 ILC cases and 25 IDC cases, respectively. In pre-menopausal women, positive nuclear staining was observed in 04/08 ILC cases and 15/25 cases of IDC with a Ki-67 positivity of 11.5±3.3% and 15.9±11.5%, respectively. In post-menopausal women, 04 ILC cases and 10 IDC cases showed nuclear staining with 15±6.9% and 16±7.1% Ki-67 positivity, respectively. Tumor size of ≤ 2 cm was observed in 03 cases of ILC and 07 of IDC with 13.9 ± 0.4% and 13.9 ± 0.4% Ki-67 positivity, respectively. Tumors of 2.1 to 5.0 cm in size were present in 02 cases of ILC and 07 cases of IDC with 10.9 ± 3.2% and 14.04 ± 5.34% Ki-67 positivity, respectively. Tumors ≥ 5.1cm were seen in 03 cases of ILC and 11 cases of IDC with 14.2 ± 9.3% and 17.38 ± 11.30% Ki-67 positivity, respectively. The results were not statistically significant.

Conclusion: Ki-67 positivity showed a tendency to increase with increasing size of the tumor in breast cancers. Since the results of pre and post menopausal patients were similar, hence response to cytotoxic chemotherapy is anticipated to be equivocal in them.

KEY WORDS: *Ki-67 Positivity Index, Immunostaining, Cytotoxic Chemotherapy.*

INTRODUCTION

Breast cancer is the most common malignancy affecting both the developed and the developing nations.[1] In Pakistan,[2,3] the incidence of breast cancer is higher compared to women from neighboring countries. Although, no population based data is available here yet the cancer registry statistics in different oncology institutes and departments verify that breast cancer is the commonest female cancer in the country One in every nine females is affected with this disease.

It has also been found that Pakistani women present with metastases at a younger age with aggressive proceedings compared to western women.[3] Evidence to support this characteristic behaviour may be establish through links to genetic factors or epidemiological data of cultural norms of diet.The prognosis of breast cancer depends not only on the extent of the disease but also on the biological behavior of the tumor, which is based on physical, histologic, and immunohistochemical characteristics. Steroid receptor status, loss of differentiation, increasing proliferative activity, inactivation of tumor suppressor genes, over-expression of oncogenes, tumor size, and age are related to tumor progression and may be used to predict prognosis.[4] In addition, tumor size at the time of diagnosis predicts nodal disease, which includes axillary metastasis, simply because it also predicts lymphatic invasion.[5] Metastasis to regional lymph nodes in women with or without lymphatic invasion has been found to be more frequent in women younger than 40. Young age is also an independent prognostic factor for women with breast cancer, independent of nodal involvement and tumor size.[6]

Proliferative activity of tumor cells assessed by immunohistochemical Ki-67 expression is one of several prognostic indicators in breast cancer.[7] Carcinogenesis

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is linked to the development of proliferative abnormalities which precede the occurrence of morphological abnormalities and hence their measurement serves as useful biomarker for chemotherapy trials and prognosis.[8,9] Many markers of proliferation have been studied in breast cancer, including thymidine labeling, bromodeoxyuridine incorporation, flow cytometry, and immunostaining with the Ki-67 monoclonal antibody.[10,11,12] This antibody recognizes a nuclear antigen encoded by a gene located on chromosome 10q25.[13,14] The antigen Ki-67 is regarded as a marker for proliferating cells. It was identified as a protein which exists free or associated with DNA as evidenced by DNA digestion of cells before or after immunolabelling with Ki-67.[15] (12). As Ki-67 identifies the proliferating cells in a tumor, it reflects the percentage of dividing cells. The positivity of the cells correlates with the degree of differentiation, vascular invasion, and lymph node metastasis, and it relates inversely to the presence of steroid hormone receptors.[16]

The purpose of the study was to evaluate proliferative indices via Ki-67 labeling in invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC) of breast and to find their relationship to age of patient and size of tumor at the time of diagnosis.

MATERIALS & METHODS

This study was performed on formalin fixed paraffin embedded blocks of cases diagnosed as invasive ductal carcinoma and invasive lobular carcinoma of breast with and without lymph node involvement, in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan from October 2000 to October 2002.

Fifty cases were selected comprising of 10 cases of invasive lobular carcinoma (ILC) and 40 cases of invasive ductal carcinoma (IDC) and were subjected to immuno-staining for Ki-67 positivity. Five-micron thick sections were retrieved for H&E staining. Extra slides were prepared for immuno-staining by cutting 4µm thick sections from representative paraffin embedded blocks and were applied to already positively charged slides. Antigen retrieval was done by trypsin digestion (Zymed Cat No. 00-3003) followed by heat induced antigen recovery. Specific staining was accomplished by localizing the Ki-67 antigen with Ki-67 polyclonal antibody. The antigen/antibody complex was then identified using the LAB-SA biotinylated secondary antibody detection method (Jonstone and Thorpe, 1988). A streptaviden enzyme is then added which binds to the biotinylated secondary antibody. A substrate solution is then added that forms a coloured deposit in the presence of the enzyme that is complexed to the antigen. The location of the antigen is then revealed by the presence of the colored deposit that forms around it. Any nuclear staining was regarded as positive. Positivity index of Ki-67 was determined by counting the number of positively stained nuclei in 1000 tumor cells in at least five representative high power fields across the slide.

STATISTICAL ANALYSIS

The computer package "Microsoft Excel" was used for data feeding and "EPI-INFOR" was used for statistical analysis. The results were given in the text as number and percentage for qualitative variables and mean and standard deviation for quantitative data. To compare the difference between two means, Student t-test was employed. For the comparison of more than two means Analysis of variance (F-test) was performed. In all statistical analysis, only 'P' values less than '0.05' were considered significant.

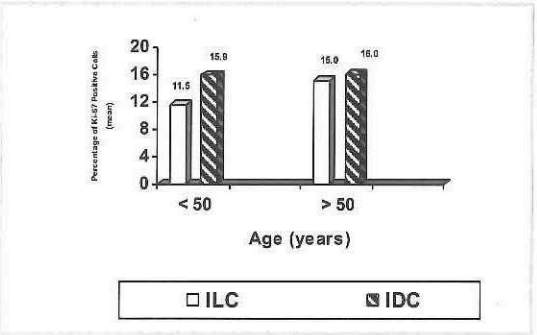
RESULTS

The youngest patient in ILC was 35 years of age and the oldest 65 years of age, with a mean age of 47.5 years. In IDC, the youngest patient found was 20 years old and the oldest was 70 years of age with a mean age of 43.95 years. All 50 cases of ILC and IDC included in this study are divided according to age at the time of diagnosis into two groups, i.e. < 50 years (premenopausal) and ≥50 years (postmenopausal) age groups. In ILC both age groups had equal number of cases, i.e. 05 cases each, comprising 10% of total cases in each group. In IDC maximum number of cases were in age group < 50 years, i.e. 26. It came out to be 52% of total cases. Age group ≥ 50 years contained 14 cases, comprising 28% of total number of cases.

Table 1: Age versus ki-67 positivity in invasive lobular carcinoma and invasive ductal carcinoma of breast

S. NO.	AGE YEARS	ILC		IDC		P VALUE
		NO. OF CASES	Ki-67 (Mean±SD)	NO. OF CASES	Ki-67 (Mean±SD)	
1	< 50	04	11.5±3.3	15	15.9 ± 11.5	> 0.05
2	≥ 50	04	15 ± 6.9	10	16 ± 7.1	> 0.05
3	Total	08		25		
4	P Value		> 0.05		> 0.05	

Figure 1: Age versus ki-67 positivity in invasive lobular carcinoma and invasive ductal carcinoma of breast



Key: ILC = Invasive Lobular Carcinoma, IDC = Invasive Ductal Carcinoma

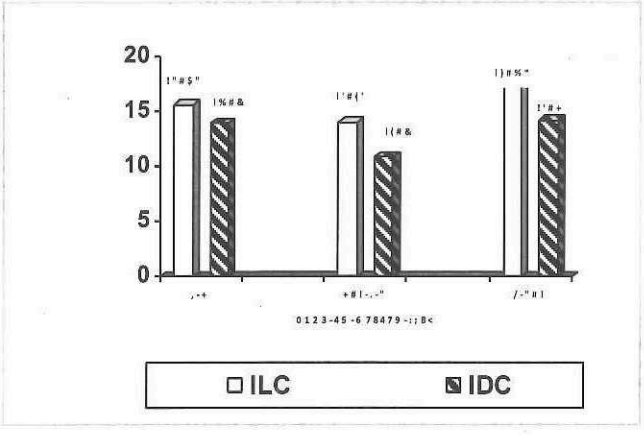
Table-1 and Figure-1 show distribution of 33 positively stained cases of ILC and IDC included in this study according to age. Group-I comprised cases <50 years of age, i.e. pre-menopausal. In ILC 04 out of a total of 08 cases were present in group-I showing a mean Ki-67 index of 11.5±3.3%. In IDC, 15 out of 25 cases were present in this group showing a mean Ki-67 index of 15.9±11.5%. Group-II comprised cases with ≥50 years of age, i.e. post-menopausal. In ILC, 04 out of a total of 08 cases were present in group-II, showing a mean Ki-67 index of 15±6.9%. In IDC, 10 out of 25 cases were present in group-II, showing a mean Ki-67 index of 16±7.1%. The results of ILC versus IDC as well as between pre- and post-menopausal age groups were found to be statistically insignificant (P>0.05).

Table-2 and Figure-2 show distribution of 50 cases of ILC and IDC of breast according to the size of tumor.

Table 2: KI-67 positivity in cases of invasive lobular carcinoma and invasive ductal carcinoma of breast versus size of tumor

S. NO.	AGE of TUMOUR	IDC		ILC		P VALUE
		NO. OF CASES	Ki-67 (Mean±SD)	NO. OF CASES	Ki-67 (Mean±SD)	
1	≤ 2	07	15.65 ± 11.65	03	13.9 ± 0.4	> 0.05
2	2.1 – 5.0	07	14.04 ± 5.34	02	10.9 ± 3.2	> 0.05
3	≥ 5.1	11	17.38 ± 11.30	03	14.2 ± 9.3	> 0.05
4	Total	25		08		
5	P Value		P > 0.05		P > 0.05	

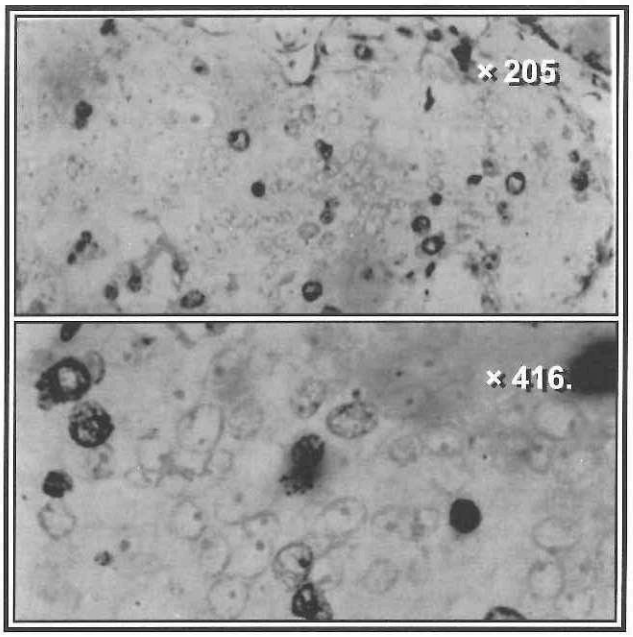
Figure 2: KI-67 positivity in cases of invasive lobular carcinoma and invasive ductal carcinoma of breast versus size of tumor



Key: ILC = Invasive Lobular Carcinoma, IDC = Invasive Ductal Carcinoma

All 50 cases had been divided into three different groups. Group-I composed of tumors equal to or less than 2 cm in size. Out of 50 cases, 03 (06%) cases were of ILC and 11 (22%) cases were of IDC with a total of 14 (28%) cases. In group-II, consisting of tumor between 2.1 to 5 cm in size, out of 50 cases 02 (04%) cases were of ILC and 14 (28%) cases were of IDC with a total number of 16 (32%) cases. Group-III reveals the tumours which were equal or more than 5.1 cm in size. Out of 50 cases 05 (10%) were of ILC and 15 (30%) were of IDC with a total number of 20 (40%) cases. Out of 50 cases 33 (66%) showed positive nuclear staining for Ki-67 antigen with 08 cases of ILC and 25 cases of IDC, respectively. In pre-menopausal women < 50 years of age, 04 out of 08 ILC cases and 15 out of 25 cases of IDC with positive nuclear staining showed a mean Ki-67 index of 11.5±3.3% and 15.9±11.5%, respectively. In cases of post-menopausal women ≥50 years of age, 04 ILC cases and 10 IDC cases showed a mean Ki-67 index of 15±6.9% and 16±7.1%, respectively. Tumors ≤ 2 cm comprising of 03 cases of ILC and 07 cases of IDC showed a mean Ki-67 index of 13.9 ± 0.4 and 13.9 ± 0.4, tumors between 2.1 to 5.0 cm comprising of 02 cases of ILC and 07 cases of IDC showed a mean Ki-67 index of 10.9 ± 3.2 and 14.04 ± 5.34, and tumors ≥ 5.1cm comprising of 03 cases of ILC and 11 cases of IDC showed a mean Ki-67 index of 14.2 ± 9.3 and 17.38 ± 11.30, respectively. These results do not show any statistical significance.

Figure 3: Photomicrograph showing invasive ductal carcinoma grade-III revealing immunohistochemical positive nuclear staning for Ki-67. Magnification × 205 and × 416.



DISCUSSION

Since axillary node involvement is the most significant and strong prognostic factor for breast cancer in women,[17] therefore, the use of systemic adjuvant chemotherapy is often determined by the presence or absence of axillary lymph node metastases.[18] Reported Variability in the incidence of nodal metastases is associated with the number of lymph nodes removed and the histopathologic methods used to find metastases. The identification of characteristics of the primary tumor that are associated with nodal metastases might cause the surgeon to perform a more extensive axillary dissection and the pathologist to use methods of examining the nodes. This increases the likelihood of finding metastatic disease. One of the most significant characteristics of primary tumors related to nodal involvement has been the presence of lymphatic invasion.[19]

Lymphatic invasion in breast cancer specimen remains occult because of pathologic sampling issues. The histological examination of greater amounts of tissue and especially that at the periphery of the lesion provides the most accurate assessment of lymphatic invasion in breast cancer. Therefore, as invasive tumors increase in size, a smaller percentage of the tumor area is histologically examined[5] Moreover, as the size increases, the peripheral areas of the tumor increase and the most relevant sites for assessment of lymphatic invasion may receive relatively less pathologic attention. In this situation, further addition of a marker, may provide better understanding of nodal metastatic potential of tumor under examination, as well as a boost to the predictable value.

The biological behavior and clinical outcome of breast cancer have been reported as predictable through several factors. These factors can envisage certain result such as proliferation index, tumor suppressor gene p53, over-expression of oncoprotein c-erbB2, tumor size, age, steroid receptors and grade of malignancy. Prediction of the aggressiveness and prognosis of the condition makes proliferation rate the most important factor among all these.[20]

Most of the previous studies attribute a controversial association between tumor size and Ki-67 labeling index.[21] In this study, tumors equal to or greater than 5.1 cm in size show high Ki-67 labeling indices, i.e. 17.38±11.3% in IDC and 14.2±9.3% in ILC compared to tumors equal to or less than 5 cm in size, though no statistically significant relationship between Ki-67 positivity and size of the tumor was achieved. Many of these have not shown a significant relationship between Ki-67 labeling and size of the tumor.[22,23,24,25] Lelle reported a weak positive relationship to tumor size, while Barzanti et al., found a significant relationship of Ki-67 positivity index to size of the tumor.[26,27]

Positron emission tomography (PET) using fluorine-18 2-deoxy-2-fluoro D-glucose (FDG) enables highly accurate differentiation between benign and malignant breast tumours. FDG uptake correlates with proliferative activity assessed by Ki-67 immunostaining. Papantoniou and workers in 2004 reported detection of a positive correlation in univariate analysis between 99mTc-(V)DMSA tumor uptake and Ki-67 expressionhese were. They also found out that these were related to the tumor size.[20] Buck and Co-workers, however, previously reported that FDG uptake correlates with proliferative activity assessed by Ki-67 immunostaining ($P<0.05$). A significant correlation with the other prognostic markers, which included tumor size, could not be demonstrated.[28]

Several studies have attributed independent association of young age with prognostic factors, such as tumor grade or proliferative measures.[29,30] Several studies have shown that Ki-67 positivity index declines or with increasing age or after menopause of the patient.[31,32,33] On the other hand, Sullivan et al., Papantoniou et al found no significant correlation between Ki-67 indices of pre-menopausal and post-menopausal women with invasive breast carcinoma. or detected any significant co-relation between age of patient and 99mTc-(V)DMSA tumor uptake and Ki-67 expression. [34,20]

In the current study both the categories of invasive breast carcinomas showed a tendency for Ki-67 positivity index to rise after menopause or with increasing age of the patient. however, results were not statistically significant. This tendency is more pronounced in invasive lobular carcinomas than in invasive ductal carcinomas which can be explained at least in cases of invasive ductal carcinomas by the fact that the maximum number of cases which were included in the age group equal to or more than fifty years were grade-III invasive carcinomas, i.e. 07 cases out of a total of 10 cases in this group. These poorly differentiated high-grade tumors have higher Ki-67 indices as compared to low-grade tumors.

CONCLUSION

Ki-67 positivity shows a tendency to increase with increasing size of the tumor in both in invasive lobular carcinoma and invasive ductal carcinoma of breast. No significant difference was observed in pre and post menopausal patients hence the response to chemotherapy would expected to be equivocal.

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