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Original Article

EXPRESSION OF Ki67 IN ER+ PR+ HER2- & ER- PR- HER2- BREAST CANCER PATIENTS

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Abstract

Background: Carcinoma of the breast is one of the most common malignancies in women worldwide.

Objective: The current study was conducted to evaluate the role of Ki-67 as a prognostic marker in two definite groups of breast cancer patients (ER+ve, PR+ve, HER-ve & triple negative) in Bangladesh perspective.

Methords: Sixty nine female breast cancer patients operated at the surgical oncology department of National Institute of Cancer Research & Hospital were selected by non-probability sampling method and operated specimens were sent for immunohistochemical study of the ER, PR, Her2/neu receptors and Ki-67 protein analysis. Statistical analysis was conducted using SPSS version 17 for Windows software. P-value 0.05 or less was considered as significant.

Result: The mean age of the patients was 46.96 years with SD of ± 13.13 years. Histopathology reports revealed that 94.2% (65/69) were suffering from duct cell carcinoma (DCC) while lobular varieties were found in 2 cases. Majority of the patients with ER+, PR+ and Ki-67 +ve status were between 36-50 years of age. But for Her2/neu positive cases most of patients were above 50 years of age. In Luminal A category cancer patients 69.7% showed positive Ki-67 expression but in case of triple negative cases this percentage was 87.5%. However, this difference was not statistically significant.

Conclusion: Scores based upon staining of ER, PR, Her2/neu and Ki-67 collectively known as IHC4 which can be used as prognostic markers in breast cancer patients.

Key words: Breast cancer, Ki-67, Oestrogen and progesterone receptor status, prognosis of breast cancer

Introduction

Breast cancer has the propensity to recur after many years of initial treatment. The exact cause for such tumor recurrence is still unknown1. Biomarkers such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and proliferation marker Ki-67 have been used for several years to predict the prognosis of breast cancer and to guide its therapy. The biological importance of

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these established markers has been reinforced over the past decade by the results from genomic classification. DNA microarray profiling studies of breast tumors has identified distinct subtypes of breast carcinomas that are associated with different clinical outcomes[2]. On the basis of gene analysis the breast tumors have been categorized into five groups: luminal A; luminal B; HER2 over expressing (HER2+); normal breast-like, and basal-like[3]. The luminal A and B subtypes are ER-positive, and luminal B is associated with a relatively worse outcome. Both HER2+ and basal-like breast cancers have poor outcomes[4].

The introduction of new genetic tests has now emphasized the role of proliferative genes, including Ki-67, as prognostic and predictive markers. Cheang et al. described an immunopanel of ER, PR, HER2, and Ki-67 that can segregate the luminal A and B subtypes[5]. Luminal B breast cancers with Ki-67 levels of at least 14% had a worse prognosis for both breast cancer recurrence and survival compared with luminal A tumors with Ki-67 levels of less than 14%[5]. HER2 is a member of the Erb family that plays an important role in promoting oncogenic transformation and tumor growth[6]. The tumors of approximately 25%–30% of the patients with breast cancer over express HER2 protein, and this overexpression is correlated with a poor clinical outcome[7]. The HER2 receptor has become important as a target for antibody-based therapy with trastuzumab. In addition to the treatment of the metastatic disease, adjuvant treatment of primary HER2-positive breast cancers with trastuzumab has been shown to markedly improve the outcome of the patients[8]. In patients with metastatic disease, selection for therapy with trastuzumab has traditionally been based on the HER2 status of the primary tumor. Ki-67 is a nuclear non-histone protein and an antigen associated with cell proliferation. It was identified after immunization of mice with Hodgkin's lymphoma[9]. The murine monoclonal antibody Ki-67 reacts with a human nuclear antigen that is expressed in G1, S, G2, and mitosis, but not in G0[10]. Numerous studies have shown that Ki-67 is of prognostic value in many types of malignant tumors. In breast cancer, a strong correlation has been found between the percentage of cells positive for Ki-67 and nuclear grade, age, and mitotic rate[11].

Our aim was to see the expression of Ki-67 in two definite groups of breast cancer patients (ER+ve, PR+ve, HER-ve & triple negative).

Materials and Methods

This cross-sectional observation study was carried out in the department of Surgical Oncology of NICRH from January 2014 to August 2015. The study was approved by the Ethical Review Committee of NICRH and informed consent was taken from each patient before their enrollment in the study. Sixty nine newly diagnosed female breast cancer patients were enrolled in the study. Sampling was convenient and purposive. The specimen or block of tissue was sent for immunohistochemical examination to specialized diagnostic centers.

Breast tissues from 69 patients of breast cancer were analyzed in the present study. The mean age of the patients was 46.96 years with SD of ± 13.13 years. Histopathology reports revealed that 94.2% (65/69) were suffering from duct cell carcinoma (DCC) while lobular varieties were found in 2 cases. There was one infiltrating papillary carcinoma and one ductal carcinoma in situ with Paget's disease. Distribution of the patients by ER, PR, Her2/neu and Ki-67 score by age group is presented in the table 1. Majority of the patients with ER+ve status were between 36-50 years of age. This finding was also true for PR+ and Ki-67 positive patients. But for Her2/neu positive cases most of patients were above 50 years of age. Expressions of Ki-67 score among categories are shown in table 2. In Luminal A category breast cancer patients 69.7% showed positive Ki-67 expression but in case of triple negative cases this percentage was 87.5%. However, on Fisher' Exact test this difference was not significant.

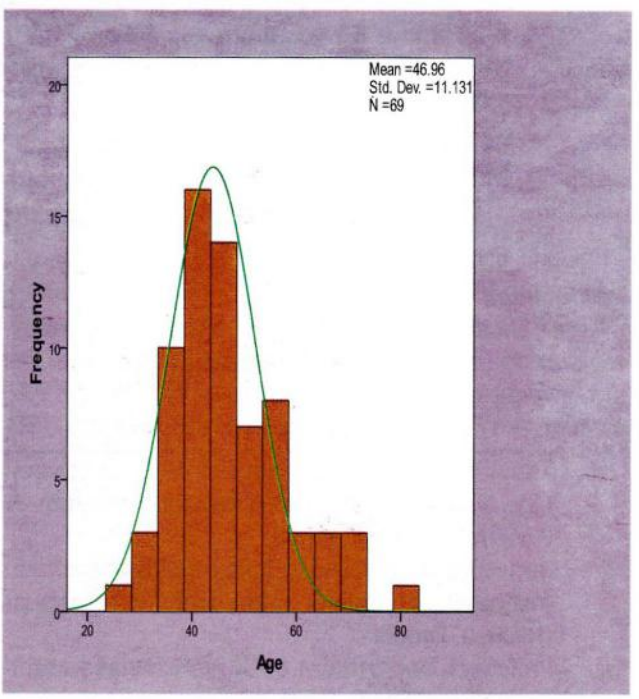

Fig. 1 Age distribution of the patients

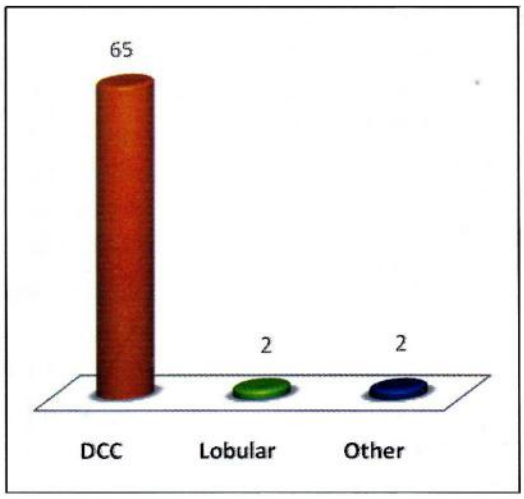

Fig. 2 Histological types of the
* Infiltrating papillary ca, ductal carcinoma in situ with Paget's disease

Table 1
Distribution of the patients by ER, PR, Her2/neu and Ki-67 score by age group

Age group (years)	ER		PR		Her2/neu		Ki-67	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
≤35	4 (10.0)	6 (20.7)	4 (10.0)	6 (20.7)	3 (16.7)	7 (13.7)	1 (5.9)	9 (17.3)
36-50	22 (55.0)	14 (44.8)	23 (57.5)	13 (44.8)	7 (38.9)	29 (56.9)	9 (52.9)	27(51.9)
>50	14 (35.0)	9 (34.5)	13 (32.5)	10 (34.5)	8 (44.4)	15 (29.4)	7 (41.2)	16 (30.8)
Total	40 (100.0)	29 (100.0)	40 (100.0)	29 (100.0)	18 (100.0)	51 (100.0)	17 (100.0)	52 (100.0)

Table 2
Expression of Ki-67 score among categories

Category	Expression of Ki-67		*p*-value *
	Negative	Positive	
Luminal A	10 (30.3)	23 (69.7)	
Triple negative	2 (12.5)	14 (87.5)	0.290
Total	12 (24.5)	37 (75.5)	

* Fisher' Exact test

Discussion

In our study Luminal A category breast cancer patients showed 69.7% positive Ki-67 expression but in triple negative cases this percentage was 87.5%. This difference was significant clinically but may be due to small sample size, no statistical significance was noted. In a study Joensuu et al. reported 43% Ki-67 positivity in primary breast cancer which is much less than our study finding (75.5%)[12]. The cause for such huge discrepancy is not clear but genetic factors may impart a role which warrants further study.

ER, PR positivity correlates with prolonged DFS and OS[13]. Proliferative rate evaluated by variety of methods including mitotic figure count, S-phase fraction, high S-phase fraction is associated with poor differentiation & poor OS. Antibodies to Ki-67 antigen can be used to determine the proliferative rate that corresponds with the S-phase fraction. The percentage of Ki-67 positivity has been used to stratify patients according to good or bad prognosis[13].

In breast cancer, prognostic tests are used to guide decision on adjuvant systemic hormonal therapy, chemotherapy and targeted therapy. This has led to large research efforts to identify novel prognostic

markers in breast cancer. The gene expression profiling and expanded immunohistochemistry tests to guide the use of adjuvant chemotherapy in breast cancer management are: MamaPrint, Oncotype Dx, IHC4 and Mammostrat. Oncotype Dx is test performed on a tumour specimen that anayses 21 gene and denotes low, intermediate and high recurrence scores that help in treatment planning14. But data from 21 gene analysis is not better than immunohistochemical (IHC4) analysis of receptor status and Ki-67 expression. Prognostic score based upon staining of ER, PR, Her2/neu and Ki-67 known collectively as IHC4, correlated with the prognostic information provided by the oncotype Dx score. In UK the cost of the Mamaprint test is £2675, the Oncotype Dx test costs £2580, IHC4 costs £200 and the Mammostat cosst between £1120 and £ 162015. In Bangladesh cost of an Oncotype Dx test is above 400,000 BDT while the receptor analyses including Ki-67 testing costs below 10,000 BDT.

Conclusion: Among the molecular subtype of breast cancer the luminal A is of better prognosis and triple negative is of worse prognosis and treatment planning is different. In case of luminal A treatment is quite satisfactory with hormone therapy. On the other hand triple negative patients are not hormone responsive but very good chemo-responsive. In this situation we can do IHC4 instead of Oncotype Dx as both tests have almost similar predictive and prognostic significance. If we popularize the test with Oncology practitioners it will be very helpful in personalized medicine and also in counseling prognostic aspects.

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