Original Research Paper

Pathology

CORRELATION OF KI-67 WITH PROGNOSTIC HORMONE RECEPTOR MARKERS IN BREAST CARCINOMA

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ABSTRACT   Ki67 is a proliferative marker which has therapeutic, predictive and prognostic value in breast carcinoma. The aim of the study was to analyse the correlation of Ki-67 labeling index with other prognostic markers like ER, PR, and HER2. This was a hospital based cross sectional comparative analysis conducted in Department of Pathology, Amala institute of Medical Sciences, Kerala, India during a period of 18 months, from January 2015 to June 2016. In total, 230 specimens of lumpectomy, mastectomy and trucut biopsies from primary breast cancer patients were enrolled in the study The immunohistochemical staining for Ki 67,Estrogen Receptor(ER), Progesterone Receptor(PR), and Human Epidermal growth factor Receptor 2(HER2) were done and scored according to specific criteria. The association between Ki 67 scores and others were evaluated.

KEYWORDS : Ki67; ER; HER2; correlation

Introduction

In 2013, there were an estimated14.9 million new cancer cases and 8.2 million cancer deaths across the world. Prostate cancer was the leading cause of cancer incidence (1.4 million) in men and breast cancer in women (1.8 million). In females breast carcinoma is the second most important cause of cancer death (464, 000 in 2013). Breast cancer is the most frequent cancer in females of India (1, 51,300) followed by cervical cancer (88,420) and oral cancer (42,390). Breast cancer deaths in India shows a comparative rise of 166% between 1990 and 2013 (1).

 In addition to the traditional pathological parameters, status of hormone receptors including estrogen receptor alpha (ER) and progesterone receptors (PR) and the human epidermal growth factor receptor 2 (HER2) amplification status, play important roles in determining the prognosis and deciding the treatment. Hormonal therapy and HER2-targeted treatments are among the most widely used targeted cancer therapies (2). Ki-67 index, a proliferation associated marker, is now widely accepted as a prognostic factor. Ki67 is a proliferative marker which has therapeutic, predictive and prognostic values in breast carcinoma, Patients with high Ki67 expression have an inferior outcome in a wide variety of malignancies including breast carcinomas(3). St Gallen guidelines recommend that decisions on systemic adjuvant therapies for breast carcinoma should incorporate ER, PR, HER2 and Ki67 assessment(4).

Currently Ki-67 is not used as a standard biomarker in breast cancer, because of the need for standardization. Also detailed guidelines are lacking. This study is intended to investigate the associations between the Ki-67 Labeling Index (LI) and other immunohistochemical characteristics in patients diagnosed with primary invasive breast cancer in our institution

Aim

The aim of the study is to analyse the correlation of Ki-67 labelling index with other prognostic markers like ER, PR, HER2

Materials and Methods

This is a hospital based study employing cross sectional comparative analysis, conducted at Department of Pathology, Amala institute of Medical Sciences, Kerala, India. Study was conducted during a period of 18 months from January 2015 to June 2016. Study was conducted after getting approval from institutional

research and ethical committees.

Study included specimens of mastectomy, trucut and lumpectomy biopsies from primary breast cancer patients received for routine evaluation in our hospital.

All non epithelial tumors and post chemotherapy or radiotherapy patients were excluded.

Procedure

A total of 230 specimens were found eligible and enrolled in the current study. According to the current CAP/ ASCO recommendations, collected specimens were fixed in10% neutral phosphate buffered (NPB) formalin. Specimens were processed and H&E stained for routine microscopic examination. In addition 4 micron thickness sections were cut from paraffin block of tumor tissue and taken on 4 glass slides coated with adhesive for IHC to detect ER, PR, HER2 over expression and Ki-67 proliferative index

Scoring of ER and PR

In each case proportion of positive stain in tumor cell nuclei (in %) and average intensity of staining (0,1+,2+,3+) were evaluated. The results were classified as negative or positive according to Allred scoring system for ER and PR. Allred score is a semi quantitative system that takes into consideration the proportion of positive cells (scored on a scale of 0-5) and staining intensity (scored on a scale of 0-3).

Scoring of Ki 67

For Ki 67, nuclear staining was scored by interpreting 3 fields in particular hotspots including a minimum of 300 cells. Score was determined regardless of staining intensity. Grading was done as negative(1+), intermediate(2+), positive(3+) and high positive(4+).

Statistical analysis:

Chi square test was done to find out association between categorical variables. Independent t-test and one way Anova were used to compare group means. Correlations between two variables were assessed using Spearman's correlation test. SPSS version 23.0 software (Licensed by Amala Institute of Medical Sciences Kerala, India)
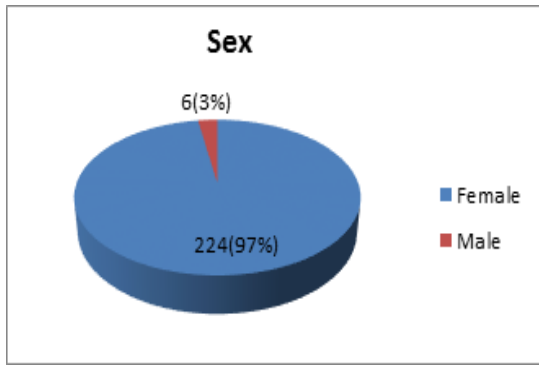
Results
Age

The mean age at diagnosis was 56.104 ±12.00 years (range 28-90). For females mean age is 55.63±11.77. Mean age in male patients taken alone is 73.66 ±5.81 (range-64-81).

Graph 1

Distribution of Age
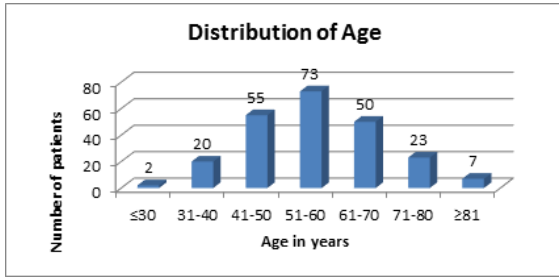
Sex

Sex ratio is 37.5:1 with male patients comprising only 6 cases (2.6 %) of the total 230 cases (28-90).
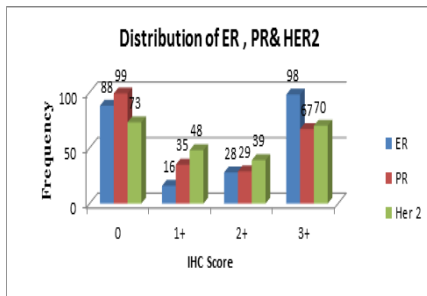

Sex

IMMUNOHISTOCHEMICAL FINDINGS - ER, PR AND HER2
ER, PR & HER2 Status

38.3 %(88 cases) were ER negative, 43% (99 cases) PR negative and 31.7 %(73 cases) HER2 negative. 61.7% cases were ER positive, 57% cases PR positive and 68.3% cases were HER2 positive. There were 98 cases in ER 3+ category, 70 cases in HER 2 3+ category and 67 cases in PR 3+ category.

Graph 3

Distribution of ER , PR& HER2

Immunohistochemical Findings - Ki 67
Distribution Patterns of Ki 67

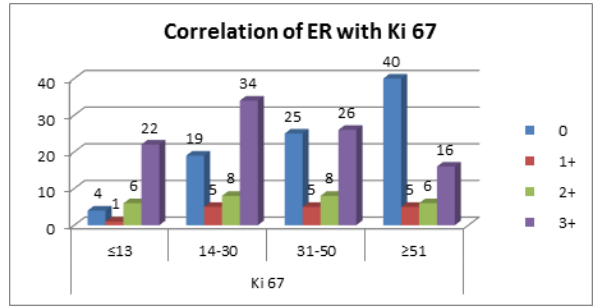The least number of cases belonged to category of low Ki 67 expression

Table 1: Distribution Patterns of Ki 67

Ki 67	Frequency	Percent
≤13	33	14.3
14-30	66	28.7
31-50	64	27.8
≥51	67	29.1
Total	230	100

Ki 67 and ER Expression

Higher levels of ER correlated with declining Ki67 score (P value - 0.0001; Kruskal Wallis test)

Graph4

Correlation of ER with Ki 67

Ki 67 and PR Expressions
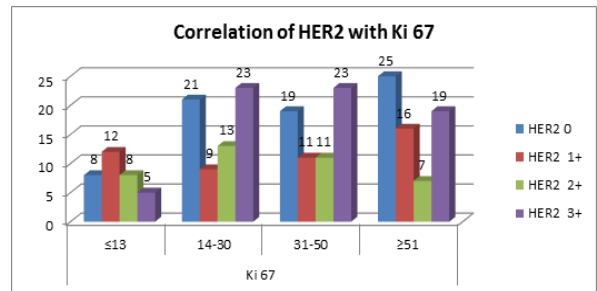
Higher levels of PR correlated with declining Ki67 score (P value - 0.0001; Kruskal Wallis test)

Correlation of PR with Ki 67
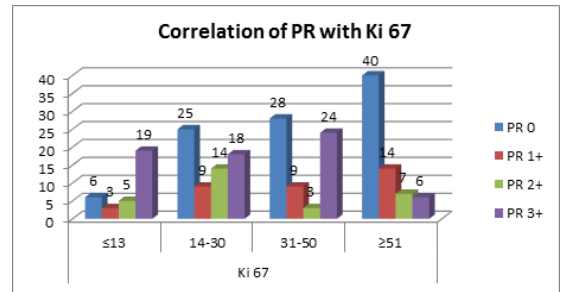
Ki 67 and HER 2 Overexpression

There was no significant correlation between Ki 67 and HER2 over expression (Pearson Chi-Square=0.116)

Correlation of HER2 with Ki 67

Discussion

Breast cancer is the most common cancer among women worldwide as well as in India.(1) There are several factors which are of prognostic, predictive and therapeutic value. Among these ER , PR ,HER 2 and Ki 67 play a crucial role in making treatment decisions and are found to be of value in this era of personalized medicine. The present study is aimed at elucidating the interrelationships of these immunohistochemical parameters with special focus on Ki67.

Age

We had a total of 230 cases with a mean age at diagnosis of 56 years(range 28-90) .The study conducted in India by Singhai et al. had patients with a median age 63 yrs(5). For females mean age is 55.63±11.77 (28-90). Mean age in male patients taken alone is 73.66 ±5.81 (range-64-81), which is almost two decades higher than females. Average age for the male breast cancer patients was 68.2 years in another study cohort constituting male patients only by Wang-Rodriguez et al.(6)

Sex

Breast cancer is the most frequent cancer in females of India. It is one among the top three causes of cancer death in women worldwide .Male breast carcinoma on the contrary, is rare, with an estimated 1 case per 1,00,000 men per year (7). Sex ratio in our study is 37.5:1 with male patients comprising only 6 cases (2.6 %).

ER, PR & HER2

In our results, majority of cases were ER positive -142 (61.7%), 88cases(38.3%) were ER negative . 131(57%) cases were PR positive , 99(43%) cases PR negative . 109(47.4%) were HER2 positive of which 39(17%) cases were borderline.121cases(52.6%) were HER2 negative.

One of the similar studies showed that the majority of cases were PR positive (62.7%), followed by ER positive (61.8%), and HER2 positive (38.2%) (68).

In another study, HER2 positive cases were the majority (30%) followed by ER and PR positive which were 15% each (126) ER showed a strong positive association with PR (Fisher's exact pvalue=0.0001). ER and PR shows an inverse correlation with HER2 in general (Fisher's exact p value=0.0001 in both). Similar results were shown in other studies also(8)

Distribution Patterns of Ki 67

Majority of cases (29.1%) had very high Ki 67 LI(>50%) , 27.8% cases showed high LI (31-50%), 28.7% cases showed intermediate expression (14-30%), and the least percentage of cases (14.3% cases) expressed low Ki67 LI(≤13%) . Thang et al. in 2015 found that 24%, 43% and 33% of the Vietnamese patients, and 37%, 35%, and 28% of the Swedish patients had tumors with low(≤15%), intermediate (16-30%) and high Ki67(>30%) indices, respectively(9)

Ki 67 and ER/PR Expressions

Higher levels of ER correlated with declining Ki67 score(P value - 0.0001;Kruskal Wallis test). Similar inverse correlation was seen between PR and Ki 67 also(P value -0.0001;Kruskal Wallis test). Many studies have shown similar results including Thang et al., Singhai et al. and Pathmanathan et al. (10) This indicates that there is an increased proliferative activity in the tumors with lower levels of ER and PR(11).

Ki 67 and HER 2 Overexpression

There was no significant correlation between Ki 67 and HER 2 in our study. Similar results were obtained by Thang et al. in Vietnamese population. HER2 over expression increased with Ki67 index in studies done by Elkablawy et al. ,Thang et al. in Swedish patients, Singhai et al., Haroon et al. ,Madani et al.(12).

Conclusion

At present ER, PR and HER 2 are the immunohistochemical markers routinely used for assessing prognosis and deciding treatment in breast carcinoma .Ki 67, the marker of proliferative activity, is a immunohistochemical marker which has got proven value in making clinical decision. Currently there is no consensus regarding its cut off and stratification.

In this study we have proven that Ki 67 is correlating with bad prognostic parameters like low ER and PR expressions, Hence its use in routine clinicopathological work up is indispensible.

We suggest that the use of Ki 67 should be made mandatory along with other immunohistochemical markers for better stratification of patients, to include them under appropriate treatment regimes and to avoid undesirable therapy.

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