Indian Journal of Pathology: Research and Practice 8(1)Jan-Feb 2019
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.8119.16

IJPRP

Original Research Article

Correlative Study of Ki67 with Other Molecular Markers in Breast Cancer in South Karnataka Population of India

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Abstract

40 females aged between 25 to 65 years old having breast cancer were studied during 2016 and August 2018. 15 (37.5%) were tobacco chewers, 4 (10%) were smokers 3 (7.5%) were alcoholic and 18 (45%) were mal-nutritious. Expression of ki67 and its histopathological correlation was found to be 4(10%) patients had IDC grade I malignancy, 22 (55%) had IDC grade II malignancy, 14 (35%) had IDC grade – III malignancy, 5 (12.5%) axillary lymph nodes had negative expression, while 35 (87.5%) expressed positivity for ki67 bio marker. The size of tumour were 4 (10%) were <2 cm and 36 (90%) with > 2 cm in the correlative study of ki67 with PR score value was 0.58, with ER score value was 0.18 and with HER-2/ neu value was 1.0 and all three correlation were statistically insignificant. This study confirmed that increasein ki67 – positivity has poorer prognosis hence ki67 biomarker plays significant role inthe prognostic value in breast cancer

Keywords: Er = Estrogen Receptor; Pr = Progesterone Receptor; Her-2 = Human Epidermal Growth Factor Receptor -2.

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Received on 17.12.2018,

Accepted on 05.01.2019

How to cite this article:

Nanjundaswamy Doddaiah. Correlative Study of Ki67 with other Molecular Markers in Breast Cancer in South Karnataka Population of India. Indian J Pathol Res Pract. 2019;8(1):106-9.

Introduction

Breast carcinoma has become the most common malignancy in female population affecting one in eight women and one of the leading causes of mortality among women globally.(1)(2)Most important prognostic factors are the size of the tumours, histological grade and lymphnode stage. Several studies have shown that, patients who have involved axillary lymph nodes have much poorer prognosis than those without modal metastasis.(3) Breast cancer is thought to derive from aberrant non – invasive breast lesions, such as atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS). However it is quite unclear how invasive breast cancer develops through these lesions. There is increasing evidence that there are several progression routes leading to invasive breast cancer

depending on histology and differentiation grades [4,5]. Hence, importance of several molecular markers in breast cancer has been considerable importance to know the status in patients during and after anticancer treatment as tumour markers, HER-2/neugene is a protooncogene mapped on chromosome 17q and encodes a transmembrane tyrosine kinase growth factor receptor that is expressed on cells of epithelial origin. In Indian women with breast cancer the HER2 / neu gene is amplified in 30% of cases [6].

The ki67 antigen first described in 1983, is a liable, non-histone nuclear protein that is tightly linked to the cell cycle and is expressed in the cell cycle and is expressed in mid-G1, S, G2 and M phases of proliferating cells but not in quiescent or resting cells of Go and early G1 phases

The expression of nuclear proliferation antigen, ki67 had been observed to reflect the rate of malignant tumours. It is associated with the development and metastasisof a variety of malignant tumours, as well as with the prognosis of patient.Many studies found ki67 to be a predictive and prognostic marker for either clinical and/or pathological response. This will be quite helpful in cases which are triple negative. Hence attempt was made to study to correlate and compare the ki67 with PR ER and HER-2/neu biomarkers.

Material and Method

Forty female patients aged between 25 to 65 years old visiting AIMS, BG Nagar 571448, Mandya (District), Karnataka were selected for study between 2016 and August 2018.

The tissue after proper labeling and fixing was processed using Automated tissue processor, paraffin embedded and then stained with Haematoxylian and Eosin for histopathological typing and grading. The histopathological grading was done according to the Nottingham modification of the Bloom Richardson grading system.Immune Histochemistry (IHC) was performed by using antibodies against ER, PR (Diagnostic Biosystem) and Her-2/neu, ki67 (Biogenex) Microwarve treatment was used as an effective method for the antigen retrieval with 10m Msodium citrate buffer at PH6. Tris buffer was used as the wash buffer and DAB (Diaminobenxene Tetrahydrochloride) was used as the chromogen. The endogenous activity was blocked by using H_20_2. After protein blocking the slides were incubated overnight with the available ER, PR Her2/neu and ki67. Primary antibodies and were conjugated with streptavidin Horse Radish peroxidase (HRP). The slides were counter stained with haemotoxylin and examined in light microscope. ER and PR was assessed semiquantitatively using MCcarty H scoring system. Ki67 labelling Index was calculated as percentage of immunostained cells divided by the total number of cells in the evaluated area of X 100 in oil immersion. O-score when no staining was given, 1– score was given when counts of positive cells < 10%, 2- when counts of positive cells were between 10-50/, 3- when count of positive cells were > 50%. Cases with score of 0 and 1 were counted as negative and 2 and 3 as positive cases

Histologically benign, previously treated or patients suffering from other cancers were excluded from the study. The duration of study was about 2 years and 8 months.

Observation and Results

The history of the patients of Breast cancer was-15 (37.5%) were tobacco chewers, 4(10%) were smokers 3 (7.5%) were alcoholic, 18 (45%) were mal-nutritious (Table 1).

Table 1: History of the Breast cancer patients (Total no of patients 40).

Sl No	Particulars	No of patients	Percentage
1	Tobacco chewers	15	37.5
2	Smokers	4	10
3	Alcoholic	3	7.5
4	Mal-nutritious	18	45

Expression of Ki67 and its (a) histopathological correlation grade I malignancy was 10% and grade-II was 55% and grade III was 35% (b) The number of positivelymphnodesobserved were 87.5% and without glands was and negative 12.5% (c) The size of the tumour more than 2cm were 90% and less than 2cm were 10% (Table 2).

Table 2: Expression of ki-67 and its histopatho-logical correlations.

Ki 67 score						Number of patients	percentage
a) Grades of Malignancy		0	1	2	3		
	I	2	0	2	0	4	10%
	II	0	5	9	8	22	55%
	III	0	3	6	5	14	35%
b) Axillary Lymphndes	No	0	3	1	1	5	12.5%

	NX	1	6	15	13	35	87.5%
c) Size of the Tumour	<2 cm	0	2	0	2	4	10%
	>-2 cm	1	6	17	12	36	90%

No= negative NX=Positive

Table 3: 1) The correlative study of scores of Ki67 with PR score was 0.58 P< 0.05 (insignificant) and with ER was 0.18 P<0.05 (insignificant with her 2/ neu score wan 1.0 P<0.05 (Insignificant) (as per the fisher exact test)

independent of tumour size lymph node status and ER expression of Ki67 correlates with other measurement of proliferation including s phase and bromide oxyuridine uptake, High ki-67 sign poor prognoses associated with good chance of clinical response to chemo therapy, but its independent significance is modest and doesnot merit measurements in most routine scenarios.

The independence of ki67 binding and lymphnode staging and tumour size together with its strong relationship with histological grade of malignancy, may ultimately allow its substitution

Table 3: Correlation between ki67 and PR, ER and Her-2/neu score.

1) Ki67			**PR Score**			**Fisher exact test**
	Negative 0-50	Weak +50-100	Moderate 200-300	Strong 200-300	Score value	p value
Negative 25.10	5 (12.5)	1 (2.5%)	1 (2.5%)	4 (10%)	0.58	p<0.05
Positive (75.30)	10 (25%)	1 (2.5%)	7 (17.5%)	11 (27.5%)		Insignaficant
2)	Ki67	ER Score			Chi square test	
Negative 10 (25%)	Negative 0-50	Weak +51-100	Moderate 100-200	Strong 200-300	Score value	p value
	3 (7.5%)	2 (5%)	1 (2.5%)	2 (5%)	0.18	p<0.05 insignificant
Positive 75%	10 (25%)	1 (2.5%)	6 (15%)	15 (37.5%)		
3) Ki67	Her 2/ neu score				Score value	p value
	Negative 0-50	Weak +50-100	Moderate 100-200	Strong 200-300		
Negative 10 (25)	6 (15%)	1 (2.51%)	1 (2.5%)	2 (5%)	1.0	p<0.05 Insignificant
Positive 30 (75%)	16 (40%)	2 (5%)	4 (10%)	8 (20%)		

Discussion

In the present study of correlation of ki67 with other molecular markers of breast cancer in southern Karnataka population 15 (37.5%) were tobacco-chewers, 4 (10%) were smokers, 3 (7.5%) were alcoholic, 18 (45%) 4 (10%) had grade-I, 22 (55%) had grade-II, 14 (35%) had grade-III, (Table 2a) 5 (12.5%) shown negative results for ki67 bio-marker and 35 (87.5%) shown positive results for ki67 biomarker (Table 2b) the size of tumours were 4 (10%) were <2cm and 36 were> 2cm in size (Table 2c) in the correlative study of ki67 with PR score value was 0.58, with ER score value was 0.18, with Her-2/neu score value was 1.0 (Fisher exact test) all these correlative values were insignificant (p<0.05) statistically. These findings were more or less in agreement with previous studies [7,8,9].

Ki67 antibody detects an antigen that is closely related to cell proliferation and thus provides a clinically useful marker for tumour to know the grades of cancer An inverse relationship between proliferative index and short term disease free survival has been noted. Strong immunostaining is most frequently seen in poorly differentiated tumours showing rates of mitotic activity, but is for the latter characteristic in a prognostic index developed to predict accurately the course of breast cancer [10].

Summary and Conclusion

The present study of correlation of ki67 with other molecular marker in breast cancer is quite useful as ki67 biomarker can diagnose, grade and prognoses as compare to other molecular bio-markers but, this study demands farther genetic, histopathological, immunological, nutritional, hormonal study because exact mechanism of ki-67 bio-marker on malignant tumour is still-unclear

This research paper was approved by ethical committee of AIMS, BG Nagar 571448, Mandya Dist, Karnataka State.

Conflict of interest : none

Funding: none

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