



Evaluation of Expression and Association of ER, PR and Ki67 Tumour Markers in Carcinoma Breast



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Abstract

Background: Several factors contribute to determine tumor prognosis and treatment strategies of breast cancer patients. Use of hormone receptor are relatively new and have proven its efficacy. **Objective:** The purpose of the present study was to evaluate the expression and association of ER, PR and Ki67 tumor markers in carcinoma breast patients. **Methodology:** In this analytical cross-sectional study, fifty-two adult cases of carcinoma breast with tru-cut biopsy underwent breast conservative surgery (BCS) or mastectomy in the Department of Surgery at Sir Salimullah Medical College and Mitford Hospital. This study excluded patients who had received neoadjuvant therapy, had recurrent breast carcinoma, had extensive metastasis, or had advanced breast carcinoma. Each participant signed an informed written consent form. Demographic information, a detailed history, and a clinical examination were all obtained. All samples were examined histopathologically and immunohistochemically. The immunohistochemistry report was used to collect biomarker data. The relationship between ER, PR, and Ki67 and histopathological findings was then investigated. SPSS 12.0 was used to analyse the collected data. **Results:** Half of the patients belonged to age group 51 to 60 years (50%). Majority respondents were from rural area (65%) and maximum were poor (57.7%). Majority respondents were T2 (48.1%) stage of tumor size followed by T1 (36%) and T3 (15.4%). According to histological grading, 44.2% had Grade II tumour, 38.5% had Grade I tumour and 17.3% had Grade III tumour. Among the respondents 80.8% had ductal carcinoma followed by decreasing order 15.4% had lobular carcinoma and 3.8% had others types of carcinoma. Regarding lymph node status, 30.8% had N1 stage lymph node involvement followed 28.8% had N2 stage lymph node involvement 26.9% had N0 stage lymph node involvement and 13.5% had N3 stage lymph node involvement. Among the respondents 65.4% had L0 and 34.6% had L1 stage of lymphatic invasion. Moreover, 78.8% had V0 stage vascular invasion and 21.2% had V1 stage vascular invasion. Of the patients, 44.2% had both ER and PR negative and 17.3% had both ER and PR positive. ER positive was found significantly higher among grade I carcinoma, PR positive was found significantly higher among grade I carcinoma and Ki67 was found significantly lower in grade I carcinoma. Among the patient's results had been found significant, ER positivity and PR positivity decline with higher lymph node staging whereas direct relation was present with Ki67. There's a significant decline in ER positivity and PR positivity with lymphatic invasion, but not with Ki67. ER and PR positivity declined with vascular invasion, whereas there was a direct correlation between Ki67 and vascular invasion. **Conclusion:** Higher grading, higher lymph node staging, and lymphovascular invasion result in a decline in ER and PR positivity, whereas Ki67 showed a direct correlation. [*Journal of National Institute of Neurosciences Bangladesh, July 2023;9(2):116-121*]

Keywords: ER; PR; Ki67; Carcinoma Breast.

Introduction

Breast cancer is the most common malignant tumor and the leading cause of death among women in the world¹. The incidence and prevalence of breast cancer in

Bangladesh is mostly unknown as we don't have any population-based cancer registries. Tumor markers are used for the detection of risk, screening, diagnosis, staging and prognosis. It can also predict the response to

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therapy, monitor treatment, detect the presence of occult metastatic disease and monitor the course of the disease. Immunohistochemical expression of tumor markers like ER, PR, Ki67 etc. help to assess the tumor status.

Estrogen has effect on target tissues by binding to fractions of cells called estrogen receptors and thus play important role in growth and development. Estrogen causes tumorigenesis by binding to the estrogen receptor which causes proliferation of mammary cells leading to increasing cell division and DNA replication, resulting in mutations. ER positive breast cancers generally have a better prognosis and responsive to hormone therapy.

Progesterone receptor (PR) is an intracellular steroid. Estrogen is necessary to induce progesterone receptors. PR along with ER expressed in various histological types of breast carcinoma. PR increasing levels directly correlate with better response, and longer survival. Ki67 is a proliferative marker. It is non histone nuclear protein that expressed in G1 through M phase of cell cycle and is not detected in resting phase of cells. Ki67 increases as cells prepare to divide into new cells. The more positive cells there are, the more quickly they are dividing and forming new cells². The knowledge about Ki67 may help in clinical decision making and prediction of breast carcinoma³.

Therefore, the present study was carried out to evaluate the expression and association of ER, PR, and Ki67 tumor markers with histopathological findings of carcinoma breast.

Methodology

Study Settings and Population: This analytical cross-sectional study was carried out in the Department of Surgery, SSMC&MH. Fifty-two diagnosed adult cases of carcinoma breast with tru-cut biopsy patient who underwent breast conservative surgery (BCS) or mastectomy in Department of Surgery at Sir Salimullah Medical College and Mitford Hospital were enrolled in this study. Patients with neoadjuvant therapy, recurrent breast carcinoma, extensive metastasis and advanced breast carcinoma were excluded from this study.

Study Procedure: Breast cancer patients undergoing BCS or mastectomy were enrolled in this study after considering inclusion-exclusion criteria. The objective of the study was discussed in details with the patients or with their attendants. Informed written consent was obtained from each of the participants. Demographic data, detailed history was taken and clinical examination was done. Histopathological examination and immunohistochemistry were done for all samples. For histopathological typing and grading, the tissue was

stained for Hematoxylin and Eosin. The Nottingham modification of the Bloom Richardson grading system were used for histopathological grading. The data about biomarkers were collected by immunohistochemistry report. The immunohistochemical assay were done on de-paraffinized formalin-fixed tissue sections (thickness 3 µm) of the samples. Then association between ER, PR and Ki67 with the histopathological findings were evaluated. Data collection was done by the investigator herself and all collected data were recorded into a data collection form.

Statistical Analysis: After collection of all the required data, these were checked, verified for consistency. Statistical significance is set as 95% confidence interval at 5% acceptable error level. Chi-square test was done whenever necessary. For all the analysis p value < 0.05 was considered statistically significant. After completion of the data collection, all were sorted and rechecked. Then data were inputted into the spread sheet of the statistical software (SPSS). Collected data was analyzed by the SPSS 12.0.

Ethical Clearance: Before starting this study, ethical clearance was taken from the ERC (Ref: SSMC/2019/261,29/12/2019) of Sir Salimullah Medical College and Mitford Hospital.

Results

A total number of 52 cases of primary breast carcinoma patients undergoing breast conservative surgery (BCS) or mastectomy conducted at the Department of Surgery in Sir Salimullah Medical College and Mitford Hospital.

Table 1: Demographic Profiles of the Study Subjects

Variables	Frequency	Percent
Age Group		
• 25 to 40 Years	5	9.6
• 41 to 50 Years	17	32.7
• 51 to 60 Years	26	50.0
• >60 Years	4	7.7
Residence		
• Rural	34	65.4
• Urban	18	34.6
Socio-economic status		
• Rich	7	13.5
• Middle	15	28.8
• Poor	30	57.7
Tumor size		
• <2cm	19	36.5
• 2-5 cm	25	48.1
• >5 cm	8	15.4

Majority respondents belonged to age group 51-60 years (50%) followed by decreasing order 41-50 years (32.7%), 25-40 years (9.6%) and >60 year (7.7%). Majority respondents were resided in rural area (65%). Majority respondents were poor (57.7%) followed by decreasing order middle class (28.8%) and rich (13.5%). Majority respondents were T2 (48.1%) stage of tumor size followed by decreasing order T1 (36%) and T3 (15.4%) (Table 1).

Table 2: Histopathological Grading of Breast Carcinoma (n=52)

	Frequency	Percent
Histopathological Grading		
Grade I	20	38.5
Grade II	23	44.2
Grade III	09	17.3
Histopathological Types		
Ductal carcinoma	42	80.8
Lobular carcinoma	08	15.4
Others	02	3.8

Among the respondents 44.2% had Grade II tumour, 38.5% had Grade I tumour and 17.3% had Grade III tumour. Among the respondents 80.8% had ductal carcinoma followed by decreasing order 15.4% had lobular carcinoma and 3.8% had others types of carcinoma (Table 2).

Table 3: Lymph Node Status of Respondents (n=52)

	Frequency	Percent
Lymph Node Status		
N0	14	26.9
N1	16	30.8
N2	15	28.8
N3	07	13.5
Stage of Lymphatic Invasion		
L0	34	65.4
L1	18	34.6
Stage of Vascular Invasion		
V0	41	78.8
V1	11	21.2

Among the respondents 30.8% had N1 stage lymph node involvement followed by decreasing order 28.8% had N2 stage lymph node involvement 26.9% had N0 stage lymph node involvement and 13.5% had N3 stage lymph node involvement. Among the respondents 65.4% had L0 and 34.6% had L1 stage of lymphatic invasion. According to stage of vascular invasion, 78.8% had V0 and 21.2% had V1 stage of vascular

invasion (Table 3).

Table 4: Combination of ER and PR among the respondents (n=52)

Combination of ER and PR	Frequency	Percent
ER-PR-	23	44.2
ER+PR+	16	30.8
ER+PR-	09	17.3
ER-PR+	04	7.7

Of the respondents, 44.2% had both ER and PR negative. In decreasing order, 30.8% of the respondents had both ER and PR positive, 17.3% had ER positive PR negative, and 7.7% had ER negative PR positive (Table 4)

Table 5: Relation of Grade of Breast Carcinoma with ER Sensitivity (n=52)

Hormone Receptor Status	Grade of Breast Carcinoma			P value
	Grade I	Grade II	Grade III	
ER				
Positive	20	5	0	0.001
Negative	0	18	9	
PR				
Positive	16	4	0	<0.001
Negative	4	19	9	
Ki67				
Positive	4	15	6	0.001
Negative	16	8	3	

p value was determined by chi-square test

The above table shows significant relation of grade of carcinoma with ER sensitivity among respondents. When the grade of carcinoma increased ER became more negative. The above table shows significant relation of grade of carcinoma with PR sensitivity among respondents. When the grade of carcinoma increased PR became more negative. The above table shows significant relation of grade of carcinoma with PR Ki67 sensitivity among respondents. When the grade of carcinoma increased Ki67 became more positive (Table 5).

Among the patient's results had been found significant, ER positivity and PR positivity decline with higher lymph node staging whereas direct relation was present with Ki67. Among the patient's results had been found significant, ER positivity and PR positivity decline with Lymphatic invasion whereas no relation was present with Ki67. The patient's ER and PR positivity declined with vascular invasion, but Ki67 was directly

Table 6: Relation of hormone receptor sensitivity with lymph node staging (n=52)

Variables	ER		PR		Ki67	
	Positive	Negative	Positive	Negative	Positive	Negative
Lymph Node Staging						
N0	14(100.0)	0(0.0)	14 (100.0)	00 (0.0)	01 (7.1)	13 (92.9)
N1	11 (68.8)	5(31.3)	02 (12.5)	14 (87.5)	09 (56.2)	07 (43.8)
N2	0(0.0)	15(100.0)	04 (26.7)	11 (73.3)	11 (73.3)	04 (26.7)
N3	0(0.0)	7(100.0)	00 (0.0)	07 (100.0)	04(57.1)	03 (42.9)
P value	<0.001		<0.001		0.009	
Lymphatic Invasion						
L0	25 (73.5)	09 (26.5)	20 (58.8)	14(41.2)	13 (38.2)	21 (61.8)
L1	00 (0.0)	18(100.0)	00 (0.0)	18(100.0)	12(66.7)	06 (33.3)
P value	<0.001		<0.001		0.096	
Vascular Invasion						
V0	25(61.0)	16(39.0)	20 (48.8)	21 (51.2)	14(34.1)	27 (65.9)
V1	0 (0.0)	11 (100.0)	0 (0.0)	11 (100.0)	11 (100.0)	0 (0.0)
P value	<0.001		0.009		<0.001	

p value determined by chi-square test

correlated with vascular invasion (Table 6).

Discussion

Breast cancer (BC) is the most common type of cancer in women and the leading cause of death in women aged 35 to 55. It is a significant burden on society. Age, tumour size, menstrual status, morphology, and lymph node status of the tumour have traditionally been the most important prognostic factors. However, research into the tumor's molecular characteristics has greatly improved the disease's prognosis⁴. At all stages of breast cancer histological grade is an independent prognostic factor. As a result, all invasive breast carcinomas should be graded. The histological grade of a tumour provides biological information about it. Proliferation (mitosis), abnormal architecture, nuclear shift, and the expression of chromosomal instability are all directly related. The Nottingham (Elston-Ellis) modification of the Patey Scarff and Bloom Richardson grading system is recommended by the World Health Organization (WHO) and the College of American Pathologists (CAP). It can be used on tissue obtained through tru-cut biopsy⁵.

According to the study, half of the patients belonged to age group 51-60 years. Dodson et al. found breast carcinoma was common in 51 to 60 years' age group and this result corresponds with our study result. Sixty-five percent of the respondents lived in a rural area in this study. Furthermore, the majority of respondents (57.7%) were poor. Most of the patients in

this study came from low socioeconomic backgrounds and traveled from rural areas in search of better medical care because the study was conducted in a government hospital. The socioeconomic, infrastructural, and infectious disease epidemics are just a few of the challenges that developing nations must overcome. However, these difficulties must not act as a barrier to effective cancer screening⁶.

In this study, majority of the patients had 44.2% Grade II tumour followed by 38.5% had Grade I tumor and 17.3% had Grade III tumour. Grade II tumour was more common among the patients diagnosed with breast carcinoma⁷. According to this study, majority of the respondents 80.8% had ductal carcinoma followed by 15.4% had lobular carcinoma and 3.8% had others types of carcinoma. In the present study of Makki in 2015, ductal carcinoma was more common than other type of carcinoma which was similar to this study result⁸.

In the study of Dodson et al., they also discovered that breast cancer patients frequently had N1 stage lymph node involvement, which is consistent with this study finding⁶.

Among the respondents 65.4% had L0 and 34.6% had L1 stage of lymphatic invasion. Moreover, 78.8% had V0 stage vascular invasion and 21.2% had V1 stage vascular invasion. Fujii et al. found less lymphatic and vascular invasion among breast carcinoma patients⁹. Regarding ER and PR, 44.2% had both ER and PR negative, 30.8% had both ER and PR positive, 17.3%

had ER positive but PR negative and 7.7% had ER negative but PR positive. Kaur et al study also found both ER & PR negative respondents comparatively more than both ER & PR positive respondents which corresponds with this study result².

The result showed significant relation between grade of carcinoma and ER. When the grade of carcinoma increased ER became more negative. Moreover, this study results also showed significant relation of grade of carcinoma with PR ($p < 0.05$). When the grade of carcinoma increased PR became more negative. Kaur et al. also found that expression of ER and PR associated with lower grade and better prognosis².

According to this present study result, there was significant relation of grade of carcinoma with Ki67. When the grade of carcinoma increased Ki67 became positive. In the study of Liu et al., they also found significant association of Ki67 biomarker with histopathological grade of carcinoma breast. Ki67 was positive in higher grade of breast carcinoma¹⁰.

This study finding also showed significant association of Lympho-vascular invasion with ER, PR and Ki67 ($p < 0.05$). When ER, PR was positive the lymphatic and vascular invasion was L0, V0 respectively and when Ki67 biomarker was negative in lymphatic and vascular invasion status was L1 and V1.

When ER and PR was positive the lymph node stage was within N0 and N1 but when Ki67 was positive lymph node stage was increased upto N3. In this study, significant relation of Lymph node staging was found with ER, PR and Ki67. Lymph node staging and lymphovascular invasion has significant relation with the Ki67 which was similar with this study result⁴.

ER, PR positive tumors respond well to hormone therapy and provide good prognosis. Whereas Ki67 increased commonly in ER, PR negative cases and carries a poor prognosis².

In this present study significant relation of, combination of hormone receptors with grade of carcinoma, lymph node staging, lymphatic invasion and vascular invasion was found ($p < 0.05$). The grade of carcinoma and lymph node staging became higher when ER, PR became negative and Ki67 became positive. The presence of lymphatic invasion and vascular invasion was more when ER, PR became negative and Ki67 became positive.

ER, PR and Ki67 had association with pathological lymph node status, high tumor grade, lymphovascular invasion, high tumor stage, and high TNM stage. Negative ER, PR and positive Ki67 expression was a

risk factor for rapid tumor recurrence and leading to poor prognosis of breast carcinoma.

Conclusion

In this study, ER and PR positivity decline with higher grading, higher lymph node staging and lymphovascular invasion whereas direct relation was present with Ki67.

Acknowledgements

None

Conflict of interest: We declare that we have no conflict of interest.

Funding agency: The author(s) received no specific funding for this work.

Contribution to authors: Kabir F designed the overall study. Akter R, Karim MA were responsible for data collection; Ullah MA, Ansary J involved in data cleaning. Karim MA conducted data analysis and interpretation. Kabir F drafted the first manuscript revised the manuscript. All authors read and approved the final manuscript.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

How to cite this article: Kabir F, Akter R, Karim MA, Ullah MA, Ansary J. Evaluation of Expression and Association of ER, PR and Ki67 Tumour Markers in Carcinoma Breast. *J Natl Inst Neurosci Bangladesh*, 2023;9(2):116-121

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Article Info

Received on: 7 April 2023
 Accepted on: 24 May 2023
 Published on: 1 July 2023

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