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Expression of p53 and ki-67 in Breast Carcinomas and Their Association with Histopathological Type, Grade, and Stage

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ABSTRACT

Background & objective: Recently many laboratories are evaluating the usefulness of determining p53, and Ki67 proliferation activities using immunohistochemical techniques in cancer. Although the available studies suggest that these factors might help make treatment decisions in cancer patients, their clinical usefulness is still controversial. The present study was undertaken to see the expression of p53 and Ki-67 in breast carcinoma and their associations with histopathological variables.

Methods: This cross-sectional analytical study was conducted in the Department of Pathology, in collaboration with the Department of Surgery, Rajshahi Medical College Hospital and the Department of Pathology, Bangobandhu Sheikh Mujib Medical University (BSMMU), Dhaka over a period of one and a half year from July 2018 to December 2019. Resected specimens of breast tissue, histopathologically confirmed as breast carcinoma, were the study population. Histological grading of breast carcinoma was done according to Nottingham modification of the Bloom-Richardson system, while staging was done using Tumor-Node-Metastasis (TNM) classification of malignant tumors, as recommended by the Union for International Cancer Control (UICC). The Ki67 and p53 expressions were assessed by immunohistochemistry. Based on the Ki67 labeling index (Ki67-LI), the patients were divided into two groups – Ki 67 > 30% (considered as over-expressed) and ≤ 30% (considered as less expressed). Likewise, p53 immuno-expression was grouped as over-expressed (score ≤ 8) and low-expressed (score > 8). The histological grade, type, and stage were then compared between patients with tumours of high and low proliferating activities to find the associations of Ki67 and p53 with tumor grade, type, and stage.

Result: In the present study, almost three-quarters (74%) of the specimen of the breast had high Ki67-LI and 26% intermediate and low Ki67-LI. The p53 was found to be highly expressed in the majority (86%) of the cases. Analyses of the association of biomarkers with histological grade, stage, and type revealed that advanced-stage breast cancers (Stage-IIIA) were more likely to be associated with high Ki67-LI than the early-stage breast cancers (Stage-IIA and Stage-IIB) (p = 0.023). Poorly differentiated (G3) carcinoma also more often tends to be associated with high Ki67-LI than with low and intermediate Ki67-LI (p = 0.066). Likewise, stage IIIA tumors and poorly differentiated carcinoma were also considerably higher in the p53 over-expressed group (23.3%) than in the low p53 expression group (14.3%) (p = 0.023).

Conclusion: The study concluded that a substantial proportion of breast carcinomas demonstrates high Ki67-LI. The p53 overexpression is also found in the majority (86%) of the breast carcinoma cases. Both Ki67 and p53 proliferation activities show their significant presence in high-grade and advanced-stage tumours.

Keywords: p53, ki-67 breast carcinoma, association, histopathologic type, grade, stage etc.

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INTRODUCTION:

Breast cancer is the second leading cause of cancer death among women after lung cancer worldwide.[1] Breast cancer accounted for 23% (1.38 million) of the total new cancer cases and 14% of the total cancer deaths (458,400) worldwide in 2008.[2] There is a gradual rise in the incidence of breast carcinoma worldwide and it accounts for about 25% of all cancer in women.[3] In Bangladesh, the prevalence of breast carcinoma is 25% in the last 5 years[4] with a total death of 7142 in 2012.[5]

Early detection and diagnostic application of immune markers and target-based therapy of breast cancer improve survival. Recently research on the biology of breast cancer has made surprising progress. To facilitate better treatment outcomes, an attempt to understand the unique biological characteristics has now been realized. Presently, the treatment strategy is not only based on the stage and classification but also on the tumor biology.[6] The study of tumor molecular characteristics has enhanced our understanding of both the risk of breast cancer recurrence and the response to therapy. Several genes implicated in breast cancer progression have been evaluated. The p53 is one of them.[3] The p53 mutations may occur in ductal carcinoma in situ (DCIS) before the development of invasive breast cancer.[7] Mutant p53 expression has a significant positive correlation with high proliferation index (MIB1),[8] increased grade values[9,10] and it shows a negative correlation with the steroid receptors status[11-13] The development and progression of breast cancer have proven to have alterations at the genomic level. The p53-negative tumors showed lower proliferation than the p53-positive tumors.[14] Most of the studies have shown an association between the p53 protein detected by immunohistochemical staining and reduced overall survival rate of breast cancer patients.[15] The detailed knowledge of the p53 mutation status and its expression predicts patients' survival,[16] response to therapy,[17] and suggests p53 as a potential target for gene therapy.[18] The proliferation has been recognized as a distinct hallmark of cancer and acts as an important determinant of cancer outcome.[19]

Immunohistochemistry for Ki-67 has also been used to determine tumor proliferation.[20] In breast cancer, a strong correlation has been found between the percentage of cells positive for Ki-67 and nuclear grade and mitotic rate.[21] Ki-67 over-expression correlates with poor disease-free survival.[22] Conversely, patients with tumors that have a very high level of proliferation have a better response to chemotherapy.[23] Ki-67 was used to predict the risk of recurrence and the excellence of chemotherapy benefits in women with breast cancer.[24]

Thus, expression of p53 and ki-67 increases with tumor grade. The grade I tumor has the lowest and the grade III tumor has the highest p53 and ki-67 values.[25] Therefore, determining Ki-67 and p53 proliferation labeling index is of utmost importance in formulating a rational treatment protocol in individual breast cancer patients which might have a positive impact on the prognosis of the disease. This study was designed to use p53 and ki-67 as immunohistochemical biomarkers and to find their association with histological types, grades and stages in breast carcinoma. The findings derived from the study will help determine the biological behavior of tumour, grade, response to chemotherapy, disease-free survival of the patients, chances of recurrence, metastasis and target-based therapy.

METHODS:

This cross-sectional analytical study was conducted in the Department of Pathology, in collaboration with the Department of Surgery, Rajshahi Medical College & Hospital, and the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka over a period of 1½ years from July 2018 to December 2019. Ethical clearance for conducting the study was taken from the Institutional Review Board (IRB) of Rajshahi Medical College, Rajshahi to carry out this study. A total of 50 resected specimens of breast tissue, histopathologically confirmed as breast carcinoma, were the study population. Patients with benign breast disease/ inflammatory breast disease, recurrent breast cancer, previously diagnosed cases of breast cancer receiving chemotherapy or

irradiation, inadequate biopsies, and/or poorly preserved specimens were excluded from the study. Histological grading of breast carcinoma was done according to Nottingham modification of the Bloom-Richardson system, while staging was done using Tumor-Node-Metastasis (TNM) classification of malignant tumors, as recommended by the Union for International Cancer Control (UICC). The Ki67 & p53 expressions were assessed by immunohistochemistry. Based on the Ki67 labeling index (Ki67-LI), the patients were divided into two groups – Ki 67 > 30% (considered as over-expressed) and ≤ 30% (considered as less expressed). Likewise, p53 immuno-expression was grouped as over-expressed (score ≥ 8) and low-expressed (score < 8). The histological grade, type, and stage were then compared between patients with tumours of high and low proliferating activities to find the associations of Ki67 and p53 with tumor grade, type, and stage.

All statistical analyses were performed using the Statistical Package for Social Science (SPSS), version 25 for Windows. Associations of Ki67-LI and p53 immuno-expression with histopathologic grading, type, and stage of breast carcinoma were determined with the help of the Chi-square (χ^2) Test. The level of significance was set at 5% and a p-value < 0.05 was considered significant.

RESULTS:

The majority (84%) of the cases were 40 or > 40 years old with the mean age of the cases being 45.1 ± 4.6 years (range: 36-53) years. The anatomical location of breast carcinoma shows that about two-thirds (66%) were diffusely located in the breast, 14% were centrally located and 10% were located in the upper outer quadrant of the breast. About one-third (32%) of the tumours were < 50 sq-cm, 34% 50 – 100 sq-cm, and another 34% >100 sq-cm (Table I). In terms of differentiation, 70% were poorly differentiated 20% moderately differentiated, & 10% well-differentiated. The majority (94%) was of ductal type carcinoma. In terms of staging Stage IIB formed the main bulk (72%) followed by Stage IIIA (22%) and IIA (6%). Nearly three-quarters (74%) of the specimen of the breast on immunohistochemistry analysis showed high Ki67 proliferation activity, 22% intermediate proliferation, and only 4% low proliferation activity. The p53 was found to be highly expressed (intensity score ≥ 8) in the majority (86%) of the cases (Table II).

Association between Ki67-LI and histologic stage of breast carcinoma demonstrates that 24.3% of the breast cancer cases with high Ki67-LI were at stage IIIA, while only 15.4% of breast cancers with low and intermediate Ki67-LI were at the same stage indicating that advanced stage breast cancers were more often associated with high Ki67-LI (p = 0.023). More than three-quarters (78.4%) of the cases with high Ki67-LI had poorly (G3) differentiated carcinoma compared to 46.2% of the cases with low and intermediate Ki67-LI indicating that high Ki67 proliferation activity is more likely to be associated with poorly differentiated carcinoma (p = 0.066). The majority (94.6%) of the cases with high Ki67-LI and 92.3% of the cases with low Ki67-LI had ductal type of breast carcinoma. There was no association between the histological type of carcinoma and Ki67-LI (p = 0.604) (Table III). Stage IIIA tumours were considerably higher in the high p53 expression group (23.3%) than that in the low p53 expression group (14.3%) (p = 0.023). Nearly three- quarters (74.4%) of high p53 expression cases were poorly differentiated carcinoma as compared to 42.9% of low p53 expression cases. Poorly-differentiated carcinomas were significantly associated with high p53 expression (p = 0.004). The histologic type of breast carcinoma was not found to be associated with p53 expression with ductal type being predominant in either group (p = 0.370) (Table IV).

Table I. Distribution of cases by their morphologic characteristics (n = 50)

Morphologic characteristics	F-value	p-value
Location		
UOQ	5	10.0
UIQ	3	6.0
LOQ	2	4.0
Central	7	14.0
Diffuse	33	66.0
Tumor size (sq. cm)		
< 50	16	32.0
50 – 100	17	34.0
>100	17	34.0

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Table II. Histopathological characteristics of the tumours and immunohistochemistry

Histological characteristics	F-value	p-value
Differentiation		
Well (G1)	5	10.0
Moderate (G2)	10	20.0
Poor (G3)	35	70.0
Histologic type		
Ductal	47	94.0
Lobular	3	6.0
TNM staging		
Stage-IIA	3	6.0
Stage-IIB	36	72.0
Stage-IIIA	11	22.0
Tumour markers		
Ki 67* (%)		
< 15 (low)	02	4.0
15 – 30 (intermediate)	11	22.0
> 30 (high)	37	74.0
p53 (intensity score)**		
< 8	07	14.0
≥ 8	43	86.0

***Median ± SEM** = 35.0 ± 1.2%; **range**: 10-45%; ****Median ±SEM** = 9 ± 0.2; **range**: 4-9

Table III. Association of TNM staging and histologic characteristics with Ki67-LI

TNM staging, histologic grading and type	Ki 67 (%)		p-value
	> 30 (n = 37)	≤ 30 (n = 13)	
TNM staging			
Stage-IIA	0(0.0)	3(23.1)	
Stage-IIB	28(75.7)	8(61.5)	0.023
Stage-IIIA	9(24.3)	2(15.4)	
Differentiation (grading)			
Well (G1)	2(5.4)	3(23.1)	
Moderate (G2)	6(16.2)	4(30.8)	0.066
Poor (G3)	29(78.4)	6(46.2)	
Type			
Moderate (G2)	35(94.6)	12(92.3)	0.604
Poor (G3)	2(5.4)	1(7.7)	

Figures in the parentheses denote the corresponding **percentage.**
*Data were analyzed using the **Chi-squared (χ^2) Test.**

Table IV. Association of staging and histologic characteristics with p53 expression

TNM staging, histologic grading and type	p53		p-value
	≥ 8 (n = 37)	< 8 (n = 13)	
TNM staging			
Stage-IIA	1(2.3)	2(28.6)	
Stage-IIB	32(74.4)	4(57.1)	0.056*
Stage-IIIA	10(23.3)	1(14.3)	
Differentiation			
Well (G1)	2(4.7)	3(42.9)	
Moderate (G2)	9(20.9)	1(14.3)	0.004*
Poor (G3)	32(74.4)	3(42.9)	
Histological types			
Ductal	41(95.3)	6(85.7)	0.370**
Lobular	2(5.4)	1(7.7)	

*Data were analyzed using the **Chi-squared (χ^2) Test**. **Data were analyzed using **Fisher's Exact Test**. Figures in the parentheses denote the corresponding **percentage**.

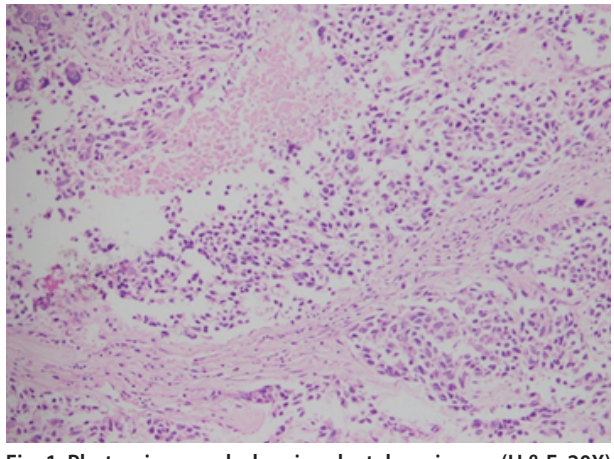

Fig. 1: Photomicrograph showing ductal carcinoma (H & E, 20X)

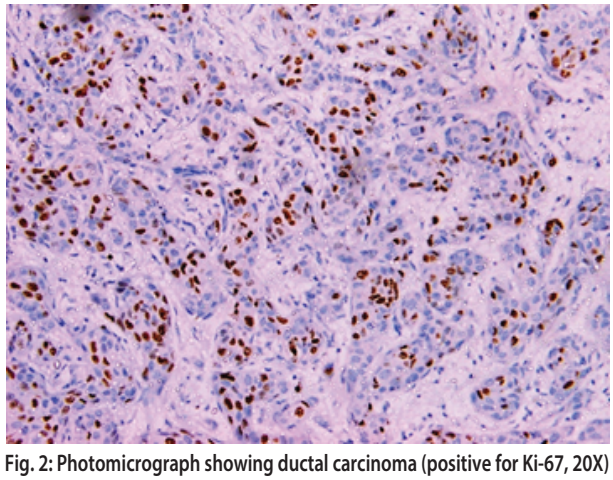

Fig. 2: Photomicrograph showing ductal carcinoma (positive for Ki-67, 20X)

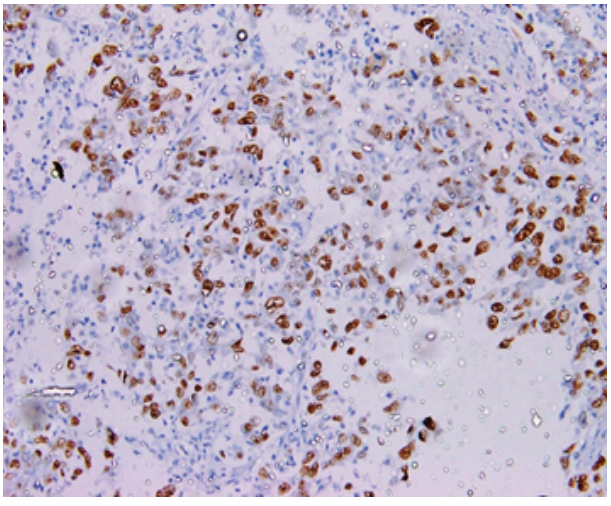

Fig. 3: Photomicrograph showing ductal carcinoma (positive for p53, 20X)

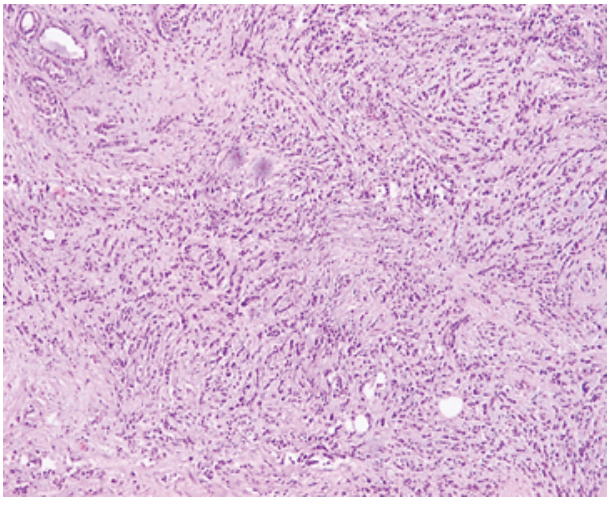

Fig. 4: Photomicrograph showing lobular carcinoma (H &E, 10x)

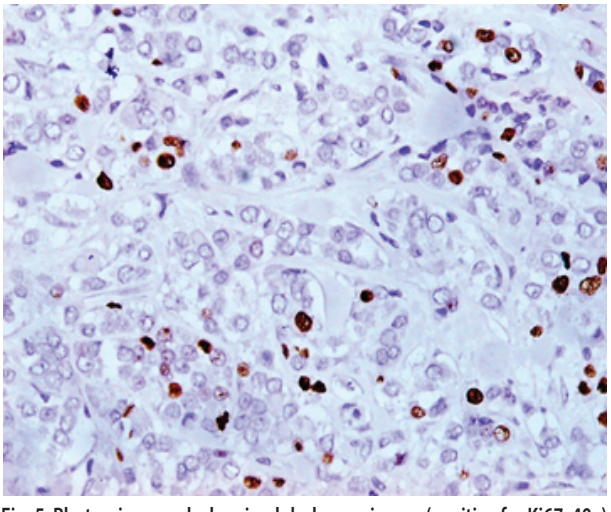

Fig. 5: Photomicrograph showing lobular carcinoma (positive for Ki67, 40x)

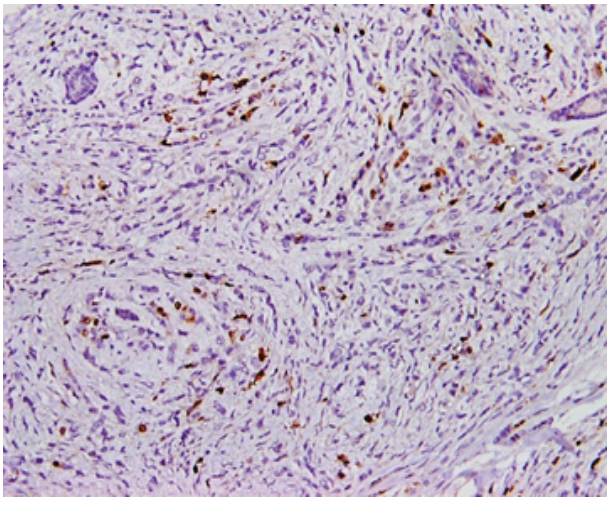

Fig. 6: Photomicrograph showing lobular carcinoma (positive for p53, 20x)

DISCUSSION:

In the present study about three-quarters (74%) of the specimen of the breast had high Ki67-LI and 26% intermediate and low Ki67-LI. The p53 was found to be highly expressed (intensity score ≥ 8) in most (86%) of the cases. Analyses of the association of biomarkers with histological grade, stage, and type revealed that advanced-stage breast cancers (Stage-IIIA) were more likely to be associated with high Ki67-LI than with the early-stage breast cancers Ki67-LI (Stage-IIA and Stage-IIB) (p=0.023). Poorly (G3) differentiated carcinoma also more often tends to be associated with high Ki67-LI than with low and intermediate Ki67-LI (p = 0.066). Likewise, stage IIIA and IIIB tumours and poorly differentiated carcinoma were also considerably higher in the p53 over-expressed group (23.3%) than that in the low p53 expression group (14.3%) (p = 0.023). Type of carcinoma, however, was not found to be associated with either Ki67-LI or p53 intensity score.

To investigate the expression of Ki67 and altered expression of the p53 gene in a series of breast cancers & their associations with clinicopathological variables, a lot of studies have been done in different parts of the world. Leonardi and associates[26] evaluated Ki67-LI in relation to tumour size, mitotic count, histological grade, nodal state as well as receptor content and altered expression of the p53 gene. A high KI67-LI (above the median value of

13.5) was associated with high grade and mitotic count, negative receptor content, and altered expression of the p53 gene.[26] In a study done by Ding et al[27] on 260 breast cancer patients in Northern China, there were significantly more patients classified histologically as a higher grade in the Ki67 high expression group than in the low expression group (grade 3, 45.1% vs. 18.7%, p=0.001) and in the p53 positive group than in the p53 negative group (grade 3, 47.4% vs. 31.7%, p=0.015). However, there was no significant difference in pTNM staging between the Ki67 high expression and low expression groups or the p53 positive and p53 negative groups.[27] Several studies have demonstrated that p53 positive is connected with the progression of carcinoma to a higher histological grade and p53 overexpression in breast cancer patients suggesting that they could be used as a prognostic biomarker for breast cancer in addition to being used as a diagnostic biomarker.[10,28-32] These findings thus strengthen the reliability of these emerging biomarkers as a predictor of breast cancer prognosis outcomes.[29] Yamashita[33] conducted a study on 506 primary invasive ductal carcinomas to see the association of biomarkers like Ki67 and p53 with clinicopathological characteristics of the tumours. Significant associations were observed between p53 overexpression and tumor size ($p = 0.0009$), and histological grade ($p < 0.0001$). Ki67 expression was also significantly associated with histological grade ($p = 0.004$).

P53 is a transcription factor that is encoded by the Tp53 gene and regulates the cell cycle as a tumor suppressor. Mutations in the p53 genes cause the formation of stabilized proteins, which accumulate and can be analyzed by immunohistochemistry.[34,35] Our finding that p53 positivity caused breast carcinoma to progress to a higher histological grade is in agreement with previous reports[3,36,37] and suggests that mutation in p53 genes has a tumor promotion role in breast cancer patients,[38] which is fortified by previous reports.[36] Ki-67 is a nuclear protein that is commonly used for the detection and quantification of proliferating cells because an increase in its expression is associated with cell growth.[39] Consistent with the observations of other investigators,[6,36,40] our observation that high Ki67 proliferation activity might have led breast carcinoma to progress to a higher histological grade has immense implications in the evaluation of prognosis of breast carcinomas. However, as the sample size was small, it is difficult to generalize the findings to the reference population.

CONCLUSION:

From the findings of the study, it can be concluded that a substantial proportion of breast carcinoma specimens demonstrate high Ki67-LI. The p53 overexpression is also found in the majority of breast carcinoma cases. Both Ki67 and p53 proliferation activities show their significant presence in high-grade (poorly differentiated) & advanced-stage tumours (Stage-IIIA). However, type of carcinoma does not have any tendency to be associated with either Ki67-LI or p53 intensity score.

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