Immunohistochemical Analysis and Molecular Subtyping of Breast Cancer

*Sharmin S,[1] Ambiya AS,[2] Hussain M,[3] Rima FA[4]

Abstract

Objectives: With recent advances in science, technology and research applications, there has been a revolution in breast cancer diagnosis and treatment modalities. Variable response to therapy and heterogeneity in tumor biology have led the urgency of sub classifying breast cancers according to the genetic basis and with the help of expression of different biomarkers. It is very important to recognize the subtypes of breast cancers to design a personalized therapy.

Methods: This descriptive cross sectional study has included 30 cases of breast cancer and has highlighted the expression of ER, PR, HER2neu, Ki67 and P53 in breast tumors which are the minimum requirement for sub-classifying them. The biomarker expression was correlated with tumor grading and staging.

Results: Luminal-A (ER+ and/or PR+, HER2-, Ki-67<14%), Luminal-B (ER+ and/or PR+, HER2-, Ki-67≥14%) , HER-2 enriched (ER-, PR-, HER2+) and triple-negative breast cancer (ER-,PR-, HER2-) subtypes accounted for 13.33%, 6.66%, 40% and 40% respectively. Her2enriched and triple-negative breast cancer cases demonstrated highest frequency with more lack of differentiation and higher proliferation.

Conclusion: Significant differences in biomarker expression was found in relation to different molecular subtypesof breast cancer.

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Keywords: Breast cancer, immunohistochemistry, molecular subtype

Introduction

Breast cancer is the most common cancer in women worldwide. It is the second leading cause of cancer mortality among women. An estimated 1.67 million new cancer cases were diagnosed in developing countries in 2012.[1] Breast cancer patients with apparently similar clinical and pathologic features can experience different disease dynamics or response to adjuvant therapies. This prognostic heterogeneity is considerable and suggests a corresponding heterogeneity of the underlying biological variables.[2] In the past decade, with the discovery of novel biomarkers, the management of breast cancer has experienced incremental improvement. Molecular subtyping of breast cancer based on the biomarkers have shown prognostic and predictive values.[3] The three predictive markers: estrogen receptor (ER), progesterone receptor (PR) and human epidermal factor-2 (HER2) are used to define the therapy of breast cancer. Recently, these predictive biomarkers are used to sub classify the breast cancers into luminal-A, luminal-B, HER-2 positive and triple negative molecular types and assess their biological behavior.

1. **Dr. Shegufta Sharmin, Assistant Professor, Department of Pathology, Shaheed Monsur Ali Medical College, Dhaka. sheguftasharmin1982@gmail.com*
2. *Dr. Ahmed Shahed e Ambiya, Resident Medical Officer, Department of Medicine, Holy Family Red Crescent Medical College Hospital, Dhaka.*
3. *Dr. Maleeha Hussain, Professor of Pathology, OSD, DGHS.*
4. *Dr. Fahmida Akter Rima, Assistant Professor, Department of Pathology, Sheikh Russel Gastro Liver Institute and Hospital, Dhaka.*

**For correspondence*

The estrogen and progesterone receptors were the first predictive molecular markers; positivity of these steroid hormone receptors generally have high sensitivity to hormone therapy. ER-negative breast cancer patients are more likely to achieve a pathologic complete response with neo-adjuvant chemotherapy than the ER-positive patients.[4] HER-2 encodes a tyrosine kinase glycoprotein, the positivity of which in breast cancer patients correlates with high sensitivity to targeted therapy.[5] Recently, the Ki67 index and p53 positivity has been proposed to be incorporated in the molecular classification system. It has been observed that Ki-67 play a pivotal role in cell proliferation and in sub classifying luminal breast cancers.[6] Patients with more than half of the tumor cells showing expression of Ki-67 are at higher risk of recurrence and high expression of this factor is associated with worse prognosis. TP53 positivity in breast cancers is significantly associated with high histologic grade and high proliferation index.[7] Recent studies suggested that p53 status might have a different predictive value for the efficacy of anthracycline/alkylating agent based chemotherapy regimen.[8] Using a panel consisting of estrogen receptor, progesterone receptor and Her2neu, female breast cancers could be classified as luminal (A or B), HER2 driven or triple-negative with prognostic significance.[9] Differential expression of Ki67 and P53 in these sub-groups helps in more accurate classification and further analysis of tumor biology.[10,11] No definite epidemiological data is available in Bangladesh regarding the molecular sub-typing and their association with the prognostic factors. This study is aimed to assess the biomarker expression and molecular sub-typing and their correlation with the prognostic factors.

*General Objectiv*e: To investigate the expression pattern of ER, PR, HER2neu, Ki67 and P53 in breast cancer and molecular profiling.

Specific Objective: To analyze the relation between ER, PR and HER2 neu with Ki67 and P53. To study the biomarker expression in relation to grade and stage of the tumor.

Methods

The present cross sectional study was carried out in the Department of Pathology, Dhaka Medical College over a period of one year from January 2016 to December 2016. The study included 40 patients. Female patients of any age group with histopathologically diagnosed cases of breast carcinoma were included in the study. Cases with missing data and poor staining quality of ER (n=2), PR (n=3), HER2 (n=2) and Ki-67 (n=3) were excluded. Patients who received chemotherapy or radiotherapy were excluded. After exclusion of cases with missing data of ER, PR, HER-2 status, P53 and Ki-67, total 30 cases of invasive breast cancer were eligible for analysis. Expression of molecular markers were determined by immunohistochemical approach on formalin fixed paraffin embedded tissue sections.

The interpretation of the reaction with antibodies against ER and PR was obtained by Allred scoring system and The Her2neu expression level was evaluated according to ASCO-CAP guideline. Proliferation level (Ki67) and P53 mutation status was reported according to the guidelines of Dako (Glostrup).

Statistical analysis of the results was obtained by window based computer software devised with Statistical Packages for Social Sciences (SPSS).

Molecular profiling: (According to St Gallen consensus-2011)

Luminal A: ER+/PR+/ HER2-/Low Ki67 index
Luminal B: ER+/PR+/HER2-/High Ki67 index
Her2neu positive: High expression of Her2 with variable expression of ER, PR
Triple negative: ER-/ PR-/ Her2neu- (12)

Results

A total of 40 cases were included in the study. Immunohistochemistry of ER, PR, HER-2neu, Ki-67 and P53 were performed in tissue blocks of these cases. But due to unavailable, incomprehensible or missing data in 10 cases, they were excluded and a total of 30 cases were studied. In the present study, it was observed that majority of the patients were in age group 41-50 years (63.3%).

Table I: Distribution of the study patients by age (n=30)

Age (in years)	Number of patients	%
31 – 40	8	26.7
41 – 50	19	63.3
51 – 60	3	10
Mean±SD		44.07
Range (min, max)	35-55	

Of the 30 malignant breast cancer cases, invasive ductal carcinoma was the most frequent (80%) diagnosis (Table II) . Among them, 14(46.66%) cases were ER positive and 16(53.33%) cases were ER negative, whereas only 9(30%) cases were PR positive and 21(70%) cases were PR negative (Table III). HER2 positive status was observed in 12(40%) cases and negative cases were 18(60%) (Table IV)

Table II: Distribution of the study patients by diagnosis (n=30)

Diagnosis	Number of patients	%
Invasive ductal carcinoma	24	80
Papillary Carcinoma	1	3.3
Cribriform Carcinoma	1	3.3
Lobular Carcinoma	1	3.3
Medullary Carcinoma	1	3.3
Micropapillary Carcinoma	1	3.3
Mucinous Carcinoma	1	3.3

Table III: Distribution of the study patients by ER and PR (n=30)

ER and PR	Number of patients	%
ER		
Positive	14	46.66
Negative	16	53.33
PR		
Positive	09	30
Negative	21	70

Table IV: Distribution of the study patients by HER2 (n=30)

HER2	Number of patients	Percentage
Positive	12	40
Negative	18	60

Table V: Distribution of Histopathological grade among various breast cancer subtypes

	Total cases	Luminal A	Luminal B	HER2+	Triple Negative	P value
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Total (n %)	30(100)	4(13.33)	2(6.66)	12(40)	12(40)	
Histological grade						
I	8(26.66)	1(25)	0	5(41.66)	1(8.33)	0.514^{ns}
II	16(53)	3(75)	2(100)	6(50)	6(50)	
III	6(20)	0	0	1(8.33)	5(41.66)	

Of the five molecular subtypes, both HER2 positive and triple negative cases were the most frequent, each having 40% distributions. This was followed by Luminal A cases, 4(13.33%). Among 4 luminal cases, 3(75%) cases were in histological Grade II and 1(25%) case was in grade I. Number of luminal B case was 2 (6.66%) and both the cases showed histological Grade-II. Of the 12(40%) HER2 positive cases, 5(41.66%) cases were in Grade-1, 6(50%) cases were in Grade-II and 1(8.33%) in Grade-III. No significant differences were found in HER2+ cases in relation to their histologic grading. Triple negative breast cancer cases accounted for 12(40%) and presented with advanced histologic grade. Of these 12 cases, there was 1(8.33%) Grade-1 tumor, 06(50%) Grade-II tumors and 05(41.66%) Grade–III tumors (Table-V).

Of the 4 subtypes in this study, HER2-positive and triple negative breast cancers showed highest tumor stage and nodal involvement. Among 12(40%) HER2-positive cases, 06(50%) cases were in stage IIa, 04(33.33%) cases were in stage IIIa and 02(16.66%) in stage IVa, whereas among the 12(40%) triple negative cases, 5(41.66%) cases were in stage IIIa , followed by 4(33.33%) cases in stage IIa, 2(16.66%) cases in stage IVa and 1(8.33%) case in stage IVb. 75% of luminal A tumors were in stage IIa, whereas 02(100%) luminal B cases were in Stage IIIa. Most of the nodal involvement was found in HER2 positive and triple negative cases (Table VI).

Table VI: Distribution of TNM stage among Breast cancer subtypes

	Total cases	Luminal A	Luminal B	HER2+	Triple Negative	P value
	N (%)	N (%)	N(%)	N (%)	N (%)	N (%)
Tumor stage						
pT (Tumor size)						
IIa	13(43.33)	3(75)	0	6(50)	4(33.33)	0.180^{ns}
IIIa	12(36.66)	1(25)	2(100)	4(33.33)	5(41.66)	
IVa	4(13.33)	0	0	2(16.66)	2(16.66)	
IVb	1(3.33)	0	0	0	1(8.33)	
IVc	1(3.33)	0	0	0	0	
pN (Lymph node involvement)						
No	10(13.33)	1(25)	0	5(41.66)	4(33.33)	0.701^{ns}
N1a	5(16.66)	1(25)	0	2(16.66)	2(16.66)	
N2a	12(40)	2(50)	1(50)	4(33.33)	5(41.66)	
N3a	3(10)	0	1(50)	1(8.33)	1(8.33)	

Table VII: Distribution of Ki67 with molecular subclasses (n=30)

Molecular Profile	Ki67				P value
	Positive (n=14)		Negative (n=16)		
	n	**%**	**n**	**%**	
Triple negative	9	64.3	3	18.8	0.036[s]
HER2+	4	28.6	8	50.0	
Luminal A	0	0.0	4	25.0	
Luminal B	1	7.14	1	6.25	

s= significant
P value reached from chi square test

In this study, Ki67 positivity was mostly observed in triple negative(64.3%) and Her2 positive(28.6%) cases (Table-VII). Ki67 positive tumors were mostly in grade-II (n=16) (Table-VIII). P53 positivity was found in 02(40%) HER2 positive cases, 02(40%) triple negative cases and in 01(20%) Luminal B case (Table-IX).

Table VIII: Correlation between Ki67 with Tumor grade (n=30**)**

Ki67	Tumor Grade						P value
	Grade I (n=8)		Grade-II (n=16)		Grade-III(n=6)		
	n	%	n	%	n	%	
<20	6	75	9	56.25	1	16.7	0.026[s]
>20	2	25	7	43.75	5	83.3	

s=significant
P value reached from chi square test

Table IX: Distribution of p53 with molecular subclasses(n=30)

Molecular Profile	P53				P value
	Positive (n=5)		Negative (n=25)		
	n	%	n	%	
Triple negative	2	40.0	10	40.0	0.199[ns]
HER2+	2	40.0	10	40.0	
Luminal A	0	0.0	4	16.0	
Luminal B	1	20.0	1	4.0	

Table X: Comparison between Ki67 with P53 (n=30)

Ki67	P53				P value
	Positive (n=5)		Negative (n=25)		
	n	%	n	%	
<20	0	0	16	64	0.015[s]
>20	5	100	9	36	

s=significant
P value reached from chi square test

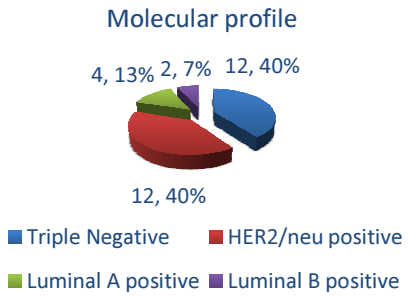

Figure 1. (Frequency distribution of molecular sub-types)

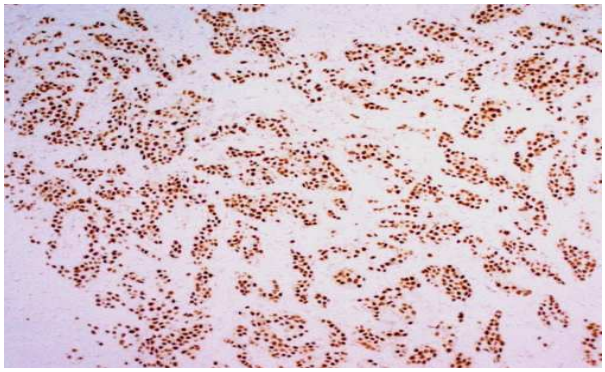

Figure 2. Estrogen receptor positive - 40x (Case-1)

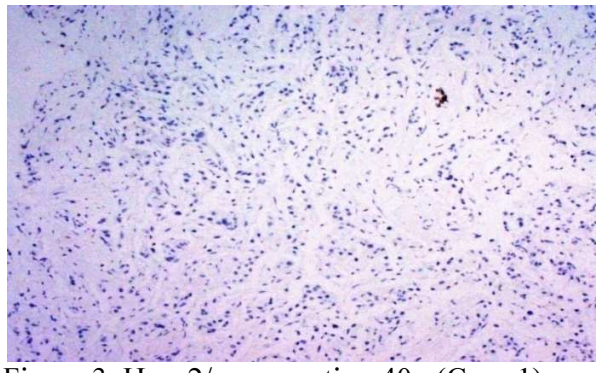

Figure 3. Her -2/neu negative-40x (Case-1)

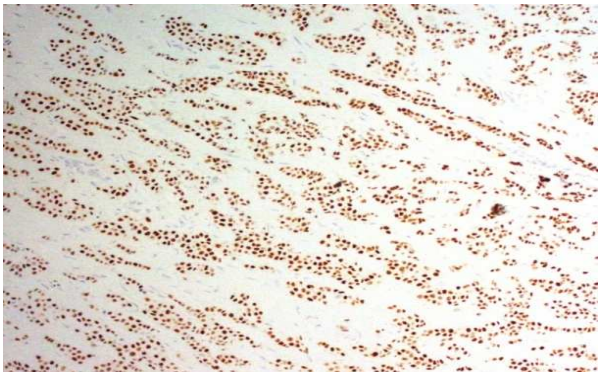

Figure 4. Progesterone receptor positive - 40x (Case-1)

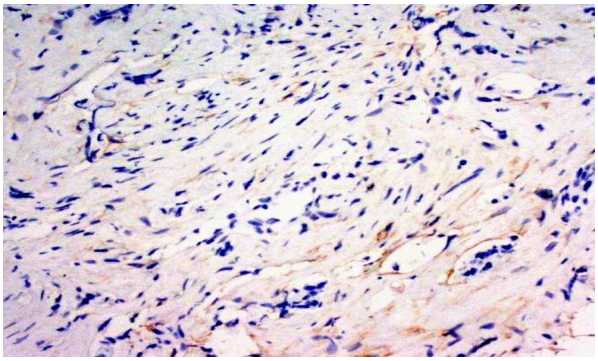

Figure 5. Negative for Ki 67- 40X (Case -1)

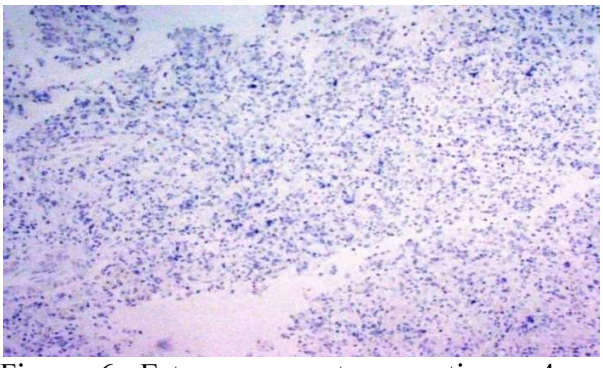

Figure 6. Estrogen receptor negative - 4x. (Case-21)

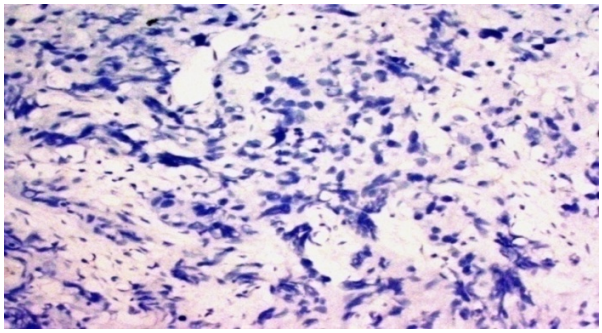

Figure 7. Progesterone receptor negative - 10x. (Case21)

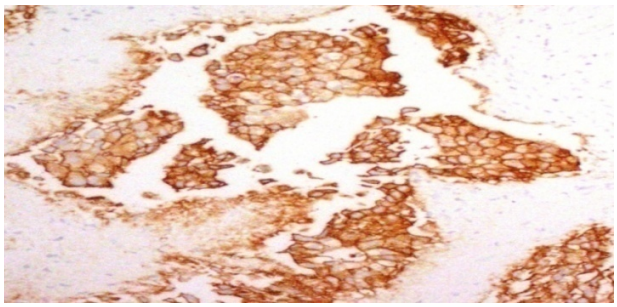

Figure 8. Her -2/neu positive-20x. (Case-21)

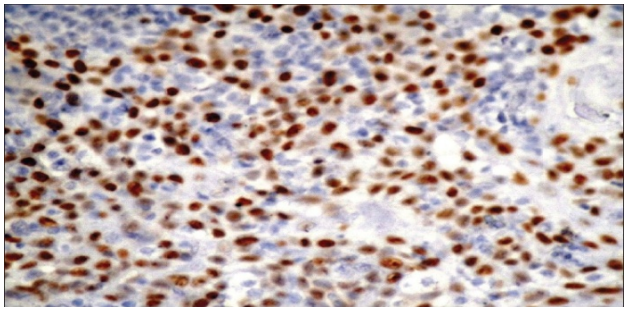

Figure 9. Ki 67 positive- 20x (Case no 21)

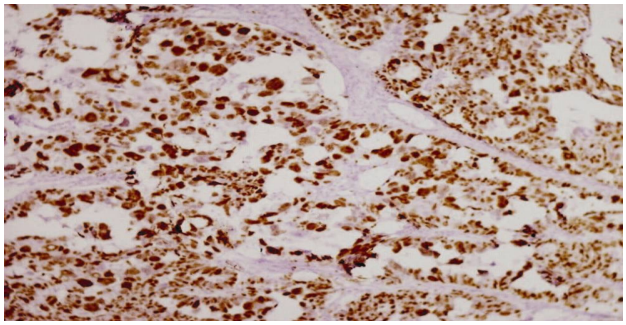

Figure 10. p53 positive- 20x (Case no 21)

Discussion

Breast cancer is a heterogeneous disease with different biological behavior, treatment response and prognosis.[13] Various factors affect the prognosis and relapse of the disease. Each type of breast cancer exhibits distinct behavior. Breast cancer was managed on the basis of histological grading and TNM staging in earlier days,[14] but now with recent advancement in gene expression profiling and immunohistochemical approaches, molecular subtyping of breast cancer contributes in the management and prognosis. It is important to identify reliable and novel prognostic or predictive markers for molecular sub-typing of breast cancers which might play an important role as an adjunct to clinical or pathologic grading and staging systems. The present study was conducted to evaluate the status of different prognostic and predictive factors in breast carcinoma and to elucidate the interrelationship between these factors in order to identify the relation of these factors with breast cancer subtypes.

In this study, the mean age of study patients was 44 years. In a statistical study conducted by the UK cancer registry, breast cancer had 2 age peaks: 1 in the 50-59 age groups and the other in 65-70 age groups.[15] Both the peak ages of breast carcinoma in UK and also in other western countries are higher and can be explained by their relatively long lifespan. In a study conducted in India, the age of breast cancer patients was less than 50 years and therefore it appears that breast cancer patients are younger in Asian countries than in European countries which again reflect lower lifespan in these regions.[16]

The most common histological type of malignancy in this study was Invasive ductal carcinoma. This finding correlates with several other studies in this region and western countries.[17,18]

The present study showed that frequencies of luminal-A, luminal B, Her2neu and triple negative subtypes were: 13.33%, 6.66%, 40%, and 40% respectively. The result is concordant with a study done in USA,[18] while discordant with a Chinese research.[19] Luminal-A subtype usually presents as low grade cancers, but in this study, 75% of luminal-A tumors were in grade II and in stage IIa. The negligence of the patients and delay in treatment may be responsible for this advanced presentation. However, low KI67 index is the main reason for lower grade and better prognosis of Luminal-A cancers.[20] Luminal-A patients respond well to hormone therapy, but are less responsive to chemotherapy.[18]

A subset of Luminal cases which were further classified as Luminal-B based on lower expression of ER, PR and high Ki67 index show high rate of recurrence and less response to hormone therapy.[21] In the present study, both Luminal B cases presented in grade II and stage IIIa with lymph node metastasis, had high Ki67 index and P53 positivity. Luminal A subtypes are more common than Luminal B.[22] But, in a related study in UK, Luminal B subtype was more prevalent (66%) than Luminal A (34%).[23]

In the study, we observed that almost all triple negative breast cancers were classified as grade II and III and most were in stage III or worse with lymph node involvement. This result is in agreement with recent observations that triple negative tumors are more aggressive (24). These tumors are strongly associated with recurrence and distant metastasis in comparison to other subtypes.[25]

In comparison to triple negative cases, the Her2 positive cases showed less aggressiveness and a good proportion were in lower grade, but lymph node involvement and staging was almost similar as triple negative cases. Several studies show the amplification of Her2 is associated with poor prognosis and Her2 positive disease appears to be sensitive to Anthracycline and the risk of recurrence in these tumors can be reduced by adjuvant Trastuzumab.[26] These Her2 positive patients in this study needs further investigation for definite conclusion.

The Ki67 reflects the proliferation rate in malignant cells. In this study, Ki67 positivity was mostly observed in triple negative(64.3%) and Her2 positive (28.6%) cases. In several studies, Ki 67 was considered as a good prognostic indicator as high ki67 influences tumor progression.[27] In a recent study in Iran, High Ki67 index was associated with large size and high grade of tumor and lymph node involvement.[28]

TP53, which is a tumor suppressor gene and plays a key role in cell cycle checkpoint control, was also found to be significantly associated with high Ki67, but did not show significant difference in distribution in different molecular subtypes in our study. Various reports have outlined the role of P53 in the progression of breast carcinoma.[29] In a study by Linda et al, 2013 states that a combined high score of Ki67 with TP53 is indicative of poorer prognosis.[30]

The present study shows that in addition to ER, PR, and Her2neu biomarkers, Ki-67 and P53 can be used as prognostic and predictive markers. However, breast cancers with P53 and Her2 positivity needs further evaluation to assess their biological behavior. In addition to traditional prognostic factors including tumor grade and TNM staging, molecular subtypes may aid clinical practice and research into breast cancer. Different molecular subtypes with different prognosis

and therapeutic options are therefore essential for breast cancer management.

Conclusion

The study has revealed that in addition to ER, PR, and Her2neu biomarkers, Ki 67 and P53 can be used as prognostic and predictive markers.However, breast cancers with P53 and Her2 positivity needs further evaluation to assess their biological behavior.

Limitation

Due to poor funding, the sample size couldnot be increased. We strongly recommend a large study sample for accurate evaluation of molecular subtypes of breast cancer in our country.

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