Original Article

Study on Estrogen Receptor, Progesterone Receptor and HER-2/ neu Expression Pattern by Immunohistochemistry in 87 cases of Invasive Breast Cancer

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Abstract

Background: *Now a day's determination of estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu expression pattern by immunohistochemistry in invasive breast cancer have become the standard procedure for breast cancer management.* ***Objective:*** *To see the expression pattern of estrogen receptor, progesterone receptor and HER-2/neu in Bangladeshi women with invasive breast carcinoma.* ***Method:*** *This cross sectional study was performed in 87 cases of invasive breast cancer. Estrogen receptor (ER), Progesterone receptor (PR) and HER-2/neu expression pattern were assessed by immunohistochemistry using monoclonal antibodies for detecting estrogen and progesterone receptors, and polyclonal antibody for detecting HER-2/neu.* ***Results:*** *All the cases were graded according to Bloom-Richardson grading system. Of those, Grade I tumour was 18 (20.69%), Grade II tumour was 58 (66.67%) and Grade III tumour was 11(12.64%). Both ER and PR positive reactivity were same and it was found 65 (74.71%) and HER-2/neu reactivity pattern were found negative in 59 (67.82%) cases and positive in 28 (32.18%) cases. A statistically significant correlation was found between the expression of ER and low grade tumour (p=0.011) and combined estrogen and progesterone receptor positive reactivity with low grade tumour (p=0.002).*

Conclusion: *ER, PR and HER-2/neu expression do not correlated with each other, so it is recommended that each test should be independently determined by immunohistochemistry in all cases of invasive breast cancer. All equivocal cases of HER-2/neu (score 2+) should be analyzed by FISH technique to find out the percentage of real score.*

Key words: *Immunohistochemistry (IHC), Estrogen receptor (ER), Progesterone receptor (PR), HER-2/neu.*

Introduction

Carcinoma of the breast is the commonest malignancy of females all over the world and second leading cause of death due to cancer among females. Several histopathological features have prognostic significance in breast cancer like cancer subtypes, tumour grade, estrogen and progesterone receptor status, HER-2/neu expression, proliferation marker (Ki 67) and DNA content[1]. Analysis of estrogen receptor (ER) and progesterone receptor (PR) status has become the standard procedure for patient's care in breast cancer treatment. Particularly estrogen receptor (ER) content correlated more with prolonged disease-free survival rate and has increased response to endocrine therapy[2]. Biopsy specimen of breast cancer should be evaluated for hormone receptors status. If any of these two receptors expression is found, a response to hormonal therapy is expected. The more the estrogen or progesterone receptors present on those cells the more chance to result on effective hormonal therapy. The goal of therapy is to starve the breast cancer cells of the hormone they thrive on, which is estrogen[3].

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Analysis of PR expression is generally reported with ER expression. PR status is independently associated with disease-free and overall survival. ER positive/PR positive tumours have a better prognosis than ER positive/PR negative tumours. But ER positive/PR negative and ER negative /PR positive tumours have a better prognosis than ER negative/PR negative tumours[4].

HER-2/neu oncogene encodes an 185kDa transmembrane protein and is expressed at low levels in a variety of normal epithelium including breast duct epithelium. HER 2/neu is over expressed and amplified in 20-30% of invasive breast cancers. In approximately 90% of the cases, protein over expression reflects amplification of the HER-2/neu gene located on chromosome[17] (17q21), (FISH)[5].

HER-2/neu over expression is associated with increase disease recurrence, metastasis and shortens survival. The over expression of HER-2/neu protein and amplification of the HER-2/neu gene is also associated with poor prognostic tumour characteristics such as high histological grade, high proliferative index, negative or lower ER expression and p53 mutation. HER-2/neu status along with ER/PR status are considered together to give any adjuvant systemic therapy[6]. HER-2/neu over expression has therapeutic implication in invasive breast cancer. Trastuzumab (Herceptin), a monoclonal antibody against the p185 protein has therapeutic efficacy in HER-2/neu over expressing tumours[7].

HER-2/neu testing should be routinely performed in patients with invasive breast cancer. Several techniques are available for the assessment of HER-2/neu status in patients with breast cancer. IHC is the most commonly practiced method. Other method is Fluorescence in situ hybridization (FISH) used to assess gene amplification. FISH should be reserved for weakly positive (2+) cases stained by IHC[6].

In western world ER, PR and HER-2/neu over expression status has been routinely practiced as an essential part in histopathology of breast cancer patients. In Bangladesh ER/PR and HER-2/neu pattern had been performed only in prescribed cases by medical and surgical oncologists. This work has been planned to explore the pattern of hormone receptors and HER-2/neu expression by immunohistochemistry in invasive breast cancer in Bangladeshi women.

Materials And Methods

This was a cross-sectional study and was performed at the department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, during the period of January'2009 to January'2011. Only histopathologically diagnosed cases of invasive carcinoma of breast who were advised for determination of hormone receptor status and Her-2/neu reactivity by IHC were included in this study. Mastectomy specimen / lumpectomy specimen or paraffin blocks were received in the laboratory. Routine tissue processing was followed in automated tissue processor. Paraffin blocks were made and sections were taken for routine Haematoxylene and Eosin (H&E) stain and Immunohistochemistry (IHC) stain. For IHC stain, sections were taken on HistoGrip coated slides. Routinely stained sections were examined under microscope and histological diagnosis was made. All cases were classified according to WHO proposed classification[8]. In cases of invasive ductal carcinoma (NOS), grading of tumour was performed by the Bloom-Richardson grading system[9].

During this study, ER/PR receptor status and HER-2/neu reactivity were detected through immunohisotichemical stain. DAKO EnVision™+/HRP System which is based on advance Labeled StreptAvidin-biotin (LSAB) method were used for visualizing the section. This method was carried out manually. Monoclonal antibodies were used for detecting estrogen and progesterone receptors, and polyclonal antibody for detecting Her-2/neu. In this study normal breast lobules /diagnosed cases of fibrocystic change were used as positive control for ER/PR receptor and prostatic tissue (diagnosed cases of nodular hyperplasia) was chosen as positive control for HER-2/neu.

Assessment of estrogen and progesterone receptor by IHC staining

Immumohistochemical assessment of the hormone receptor status of breast carcinoma was done by using the Allred Score system, which assessed both the proportion of stained cells and the intensity of staining. Only nuclear staining was interpreted as positive (Figure 3 & 4). The proportion of tumours cells showing positive reactivity and their intensity of reactivity were included in the Allred Score. The individual marking of scoring is shown in table l. Adding the proportion and intensity score gives a maximum score of 8. Tumours with total scores of three or more were reported as "positive" and zero to two score reported as "negative".

Score for proportion of positive staining Cells	Score for staining intensity
0= No nuclear staining.	0= No Staining of any nuclei at high magnification.
1= <1 % nuclear staining.	1= Weak Staining (only visible at high magnification).
2=1- 10 % nuclear staining.	2= Moderate Staining (readily visible at low magnification).
3= 10 -33 % nuclear staining.	3= Strong Staining (strikingly positive at low magnification)
4= 33 - 66 % nuclear staining.	
5= 66 -100% nuclear staining.	

Table I: Allred score system[10].

Sum of proportion score and intensity score:

Total score	**Interpretation**
Score 0 - 2	Negative
Score 3 - 8	Positive

HER-2/neu (c-erbB-2) over expression was measured by immunohistochemistry. A strong complete membrane staining is observed in >10 % of the tumour cells reported as "Positive" (Figure 6). HER-2/neu scoring system and its interpretation are shown in table ll.

Score	HER -2/neu protein over expression assessment	Staining pattern
0	Negative	No staining is observed, or membrane staining is observed in <10% of the tumour cells.
1+	Negative	A faint/barely- perceptible membrane staining is detected in>10% of tumour cells. The cells are only stained in part of the membrane.
2+	Weakly positive	A weak to moderate complete membrane staining is observed in >10 % tumour cells
3+	Positive	A strong complete membrane staining is observed in >10 % of the tumour cells.

Table II: Detailed criteria of individual score and interpretation of result of HER-2/neu status.

Observations And Results

A total of 87 cases of invasive breast cancer were included in this study. Mean age of the patients was 42.57 years. Age distribution ranged from 22 to 65 years with a Mean±SD of age 42.57±9.81 years. The median age was 42 years. The patients are divided into five age groups which are shown in table III.

Age in years	Number of cases	Percentage (%)
21 - 30	10	11.50
31 - 40	31	35.63
41 - 50	31	35.63
51 - 60	12	13.79
61 - 70	3	3.45
Total	87	100

Table III: Distribution of patients according to age.

In this study 51 (59%) patients came from rural area and 36 (41%) patients came from urban area. Some risk factors were recorded and are shown in table IV

Parameter	**Yes (%)**	**No (%)**
Menopause	39 (44.83%)	48 (55.17%)
Breast fed.	81 (93.1%)	6 (6.9%)
Contraceptive use	60 (68.97%)	27 (31.03%)
Family history of any cancer: breast = 6, others = -7, (liver-3, cervix-2, lung-1, penis-1).	13 (14.94%)	74 (85.06%)

Table IV: Frequency of several risk factors of breast cancer.

All of the 87 cases were diagnosed histopathologically as invasive ductal carcinoma (NOS). Tumour sizes were grouped into three subgroups which are shown in figure 1.

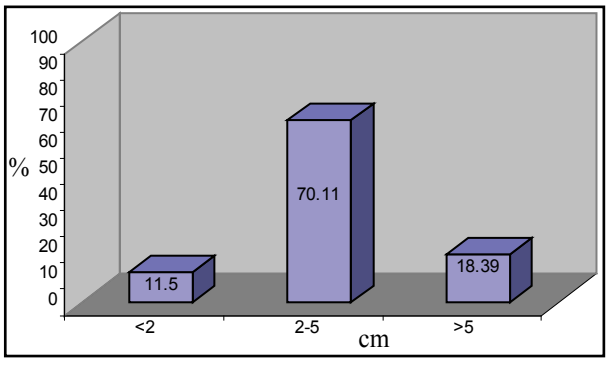

Figure 1: Frequency of tumour size (cm).

According to Bloom Richardson Grading system, 87 case of invasive ductal carcinoma (NOS) was graded into Grade I, Grade II and Grade III. Grade I tumour was 18 (20.69%), Grade II tumour was 58 (66.67%) and Grade III tumour was 11(12.64%) cases. (Figure 2).

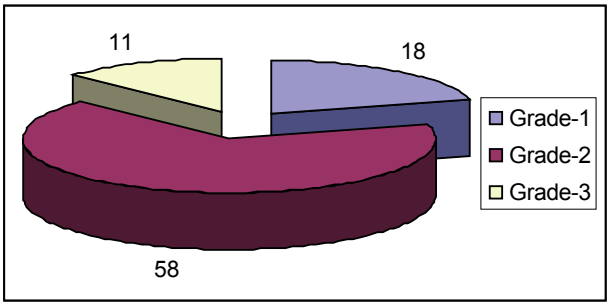

Figure 2: Frequency of tumour Grade.

Hormone receptor status

According to Allred Score criteria, both ER and PR positive reactivity were same and it was found 65 (74.71%). The patterns of ER and PR expression status are shown in table V.

Receptor	No of cases	Percentage (%)
ER (+)	65	74.71
PR (+)	65	74.71
ER (+)/PR (+)	50	57.47
ER (+)/PR (-)	15	17.24
ER (-)/PR (+)	15	17.24
ER (-)/PR (-)	7	8.05

ER= estrogen receptor; PR= progesterone receptor.

Table V: Status of ER and PR in 87 cases of invasive ductal carcinoma.

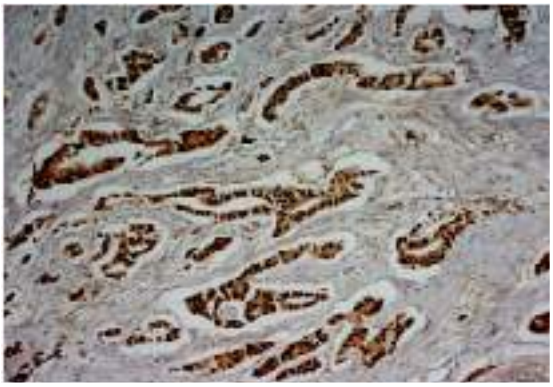

Figure 3: Estrogen receptor positive reactivity (IHC stain, X 100)

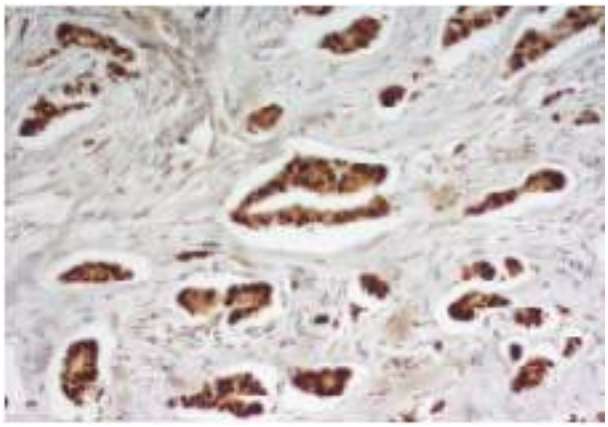

Figure 4: Progesterone receptor positive reactivity.

HER-2/neu reactivity patterns

HER-2/neu reactivity pattern were found negative (score 0, 1+, 2+), 59 (67.82%) cases and positive (score 3+), 28 (32.18%) cases, shown in the pie diagram (Figure 5).

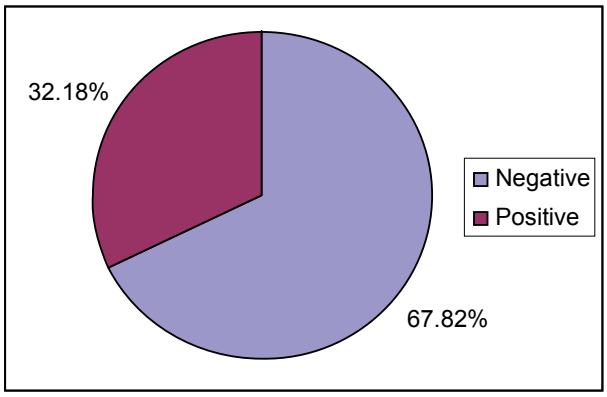

Figure 5: Percentage of HER-2/neu reactivity pattern

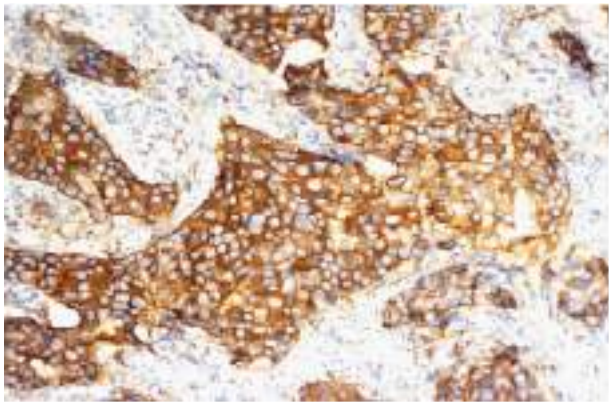

Figure 6: HER-2/neu over expression (score3+) (IHC stain, X 100).

The combined profile of hormone receptors and HER-2/neu

The combined profile of hormone receptors and HER-2/neu also observed which are shown in table Vl.

Combined hormone receptors and HER-2/neu status (3+)	No	%
ER (+) PR (+) HER-2/neu (-)	36	41.37
ER (-) PR (-) HER-2/ neu (-)	4	4.6
ER (-) PR (-) HER-2/ neu (+)	4	4.6
ER (+) PR (-) HER-2/neu (-)	11	12.64
ER (-) PR (+) HER-2/neu (-)	8	9.2
ER (+) PR (-) HER-2/neu (+)	4	4.6
ER (-) PR (+) HER-2/neu (+)	7	8.05
ER (+) PR (+) HER-2/neu (+)	14	16.1
Total	87	100.00

ER= estrogen receptor, PR= progesterone receptor, HER= human epidermal growth factor receptor-2.

Table Vl: Combined profile of hormone receptors and HER-2/neu.

Several correlations were observed in this study. But no statistical significant correlation were found between the expression of ER and PR reactivity (p=0.292), between the expression of ER and HER-2/neu expression (p=0.123) and between the expression of PR and HER-2/neu expression (p=0.966). Low grade tumour had more tendency of having positive reactivity for estrogen receptor and Grade I tumour showed 100% positive reactivity (table Vll). A statistically significant correlation was found between the expression of ER and tumour grade (p=0.011). It was also observed that low grade tumour showed higher percentage of combined estrogen and progesterone receptor positive reactivity than higher grade tumour (table Vlll). These correlations were statistically significant (p=0.002). But no statistical significant correlations were observed between individual PR reactivity with tumour grade (p=0.21) and HER-2/neu expression with tumour grade (p=0.504).

Tumour Grade	ER (-) N (%)	ER (+) N (%)	Total
Grade I	0 (0%)	18 (100%)	18
Grade II	17 (29.31%)	41(70.61%)	58
Grade III	5 (45.45%)	6(54.55%)	11
Total	22	65	87

Table Vll: Relationship between ER expression and tumour grade.

ER/PR expression pattern	Grade -1 No (%)	Grade -II No (%)	Grade -III No (%)	Total
ER+PR+	15 (83.33%)	30 (51.72%)	5 (45.46%)	50
ER+PR -	3 (16.67%)	11(18.97%)	1(9.09%)	15
ER -PR+	0	14 (24.14%)	1(9.09%)	15
ER -PR -	0	3 (5.17%)	4 (36.36%)	7
	18 (100%)	58 (100%)	11 (100%)	87

Table Vlll: Correlation between combined hormone receptor expression and tumour grade.

No correlation was found between the expression of hormone receptor and HER-2/neu over expression with the age of the patients and tumour size. Regarding risk factors correlation between the expression of hormone receptor and HER-2/neu over expression with menopausal status, breast feeding status, contraceptive users and family history of malignancy were statistically insignificant.

Discussion

This cross-sectional study was performed to see estrogenreceptor, progesterone receptor status and HER-2/neu expression pattern in invasive breast carcinoma. A total of 87 cases of invasive breast carcinoma were included in this study. All of the cases were invasive ductal carcinoma (NOS). All of them were Bangladeshi and among them 51 (59%) cases came from rural areas and36 (41%) cases came from urban areas. In this study the mean age of the patients was 42.5 years.

Lester[11] described average age of breast cancer is 61 years for white women, 56 years for Hispanic women and 46 years for African American women. Naeem et al[1] mentioned in his study mean age of breast cancer 48.3 years in Pakistanian women. According to Ayadi et al[12] mean age of breast cancer in Tunisian women is 51.5 years while Huang et al[13] mentioned mean age 50 years in Belgium women. So mean age of breast cancer is younger than reference values described by other investigators. It requires further careful examination to determine the nature of the predisposing factors.

Estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu status vary from country to country. In the present study, individual positive reactivity of ER and PR were same which was 74.71% and HER-2/neu over expression was 32.18%. ER positive reactivity is less than the studies of Ahmed et al[14], Nadji et al[2], Huang et al[13], Narita et al[15] and Thompson et al[16] who showed ER positivity 90%, 75%, 81.1%, 82% and 79.6% respectively. On the other hand it is higher than the studies of Ivkvic-Kapicl et al[6], Farzami et al[17], Ayadi et al[12], Ratnatunga & Liyanapathirana[18] and Azizun-Nisa et al[19] who showed ER positivity 73%, 62.4%, 59.4%, 53.2% and 32.7% respectively.

Percentage of PR positive reactivity in the present study is less than the study of Ahmed et al[14] who showed 77.5% PR positive cases. My study showed higher PR positivity than the studies of Narita et al[15], Ivkvic-Kapicl et al[6], Huang et al[13], Thompson et al[16], Farzami et al[17], Nadji et al[2], Ratnatunga & Liyanapathirana[18] and Azizun-Nisa et al[19] who showed 73.6%, 66%, 64.2%,62%, 61.5%, 55%, 50% and 25.3% PR positivity respectively. The percentage of HER-2/neu over expression is lower than the studies of Kumar et al[20], Narita et al[15] and Benohr et al[21] who showed HER-2/neu over expression as 46.37%, 37.3% and 34.0% respectively. But the percentage of HER-2/neu over expression in my study is more than the studies of Azizun-Nisa et al[19], Farzami et al[17], Ivkvic-Kapicl et al[6], Ayadi et al[12], Ratnatunga & Liyanapathirana[18] and Huang et al[13] who found HER-2/neu over expression 24.7%, 21.7%, 20%, 18.1%, 14.6% and 10.9% respectively.

In the present study combined ER/PR negative pattern is very low in comparison to others. This variation may be due to variation of sample size and grading of tumour because number of high grade tumour is relatively low. Combined profile of hormone receptor together with HER-2/neu has therapeutic implication. Ratnatunga & Liyanapathirana[18] found, ER (+) PR (+) HER-2/neu (-) profile in 40.32% cases while present study showed this reactivity profile in 41.37% cases. Both studies have more or less similar expression pattern. But other variants of combined profile have wide variation between these two studies (Ratnatunga & Liyanapathirana[18] versus present). These are ER (-) PR (-) HER-2/ neu (-), (28.23% vs 4.6%), ER (-)PR (-) HER-2/neu (+), (10.48% vs 3.44%), ER (+) PR (-) HER-2/neu (-), (6.45% vs 12.64%), ER (-) PR (+) HER-2/neu (-), (4.03% vs 9.2%), ER (+) PR (-) HER-2/neu (+), (1.61% vs 4.6%), ER (-) PR (+) HER-2/neu (+), (1.61% vs 8.05%) and ER (+) PR (+) HER-2/neu (+) (0.81% vs 16.1%).

In this study correlation between estrogen receptors and progesterone receptors expression status was evaluated. 50(76.92%) cases of ER positive tumours were associated with PR positivity but this correlation was not statistically significant ($p > 0.05$). Ratnatunga & Liyanapathirana[18] showed significant correlation ($p < 0.05$) between these two variables. This discrepancy may be due to relatively small sample size of my study. In the present study, HER-2/neu expression was compared with ER and PR expression. Her-2/neu over expression in ER negative cases was 45.45% which was higher than ER positive cases (27.67%). Her-2/neu over expression in PR negative cases 31.82% and in PR positive cases 32.31% which were more or less similar.

These differences are not statistically significant ($p > .05$). Some of the earlier studies showed inverse correlation between the ER and PR expression and HER-2/neu over expression (Ayadi et al[12], Ratnatunga & Liyanapathirana[18], Naeem et al[1] and Huang et al[13]). But Farzami et al[17] and Eisenberg et al[22] did not observe this inverse correlation. Hormone receptors and HER-2/neu expression correlated with several factors such as age, tumour grade and tumour size.

In the previous studies most of the investigators (Ayadi et al[12], Ratnatunga & Liyanapathirana[18], Farzami et al[17] and Huang et al[13]) found that estrogen and progesterone receptor status significantly associated with low grade tumour ($p<0.05$). In this study 100% Grade I tumour and 70.69% Grade II tumour showed positive reactivity for estrogen receptor (ER) which is statistically significant ($p=0.011$) as others.

In case of progesterone receptor, tumour grade did not show statistically significant ($p>0.05$) correlation with positive reactivity which was dissimilar to the observations of other investigators. Combined ER/PR expression was correlated with tumour grade in the present study. 83.33% Grade I tumour, 51.72% Grade II tumour and 45.46% Grade III tumour showed combined ER+PR+ reactivity pattern. On the other hand no Grade I tumour showed ER-PR- reactivity pattern, 5.17% in Grade II tumour and 36.36% in Grade III tumour showed combined negative (ER-PR-) reactivity pattern. Correlations between tumour grade and combined ER/PR receptor expression were statistically significant ($p< 0.05$).

In the present study, 22.22% of Grade I tumour, 36.21% of Grade II tumour and 27.27% of Grade III tumours showed over expression for HER-2/neu and their association were not statistically significant ($p>0.05$). In the study of Ivkovic-Kapicl et al[6], high grade tumour were more likely to demonstrate HER-2/neu over expression than the lower grade tumour ($p<0.01$). Similar findings were also reported by other authors (Huang et al[13], Ratnatunga & Liyanapathirana[18] and Lal et al[23]). This discrepancy may be due to less number of higher grade tumours included in the present study.

Conclusion

The issue of the breast cancer care development is certainly related with a number of diagnostics approaches in which immunohistochemical determination of ER, PR and HER-2/neu play certain role. Presently surgical oncologists in Bangladesh face major challenges due to lack of information about hormone receptors and HER-2/neu expression status. As ER, PR and HER-2/neu expression do not correlated with each other, so it is recommended that each test should be independently determined by immunohistochemistry in all cases of invasive breast cancer. All equivocal cases of HER-2/neu (score 2+) should be analyzed by FISH technique to find out the percentage of real score.

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