Original Paper

Immunohistochemical Staining of Estrogen Receptor and Human Epidermal Growth Factor Receptor 2 in Carcinoma Breast in Bangladeshi Women

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Abstract

Introduction: Breast cancer is the most common cause of cancer death among women. Estrogen Receptor (ER), Progesterone Receptor (PR) and human epidermal growth factor receptor 2 (Her-2/neu) status are used as an important prognostic marker for breast cancer.

Objectives: The aim of this study was to evaluate ER and Her-2 in breast carcinoma among Bangladeshi women and to co-relate with other prognostic parameters.

Materials and Methods: The study was carried out in a tertiary level hospital in Bangladesh during the period of January 2010 to December 2011. Total 100 histologically proved breast carcinoma cases were included in the study. Immunostaining for ER and Her-2/neu were carried out. Quick scoring of ER and expression of Her-2/neu were correlated with histomorphological parameters.

Results: Among 100 cases 93 were ductal carcinoma and 07 were non ductal. Among all, 38% show ER positive (score- 3-8) while 62% are ER negative (score 0-2). Her-2/neu was over expressed in 30%, 22% were borderline and 48% were negative. There were inverse relationship between ER positivity and Her-2/neu overexpression. ER positivity was related with lower histological grade, while Her-2/neu overexpression was related with higher histological grade.

Conclusions: ER positivity in Breast carcinoma patients in Bangladesh is lower in comparison to western literature while Her-2/neu overexpression rate is quite high like other Asian countries.

Key-words: Carcinoma breast, Estrogen receptor, Her-2/neu, Immunostaining, Diaminobenzidine (DAB).

Introduction

Of the 10 million new cancer cases diagnosed each year, 4.7 million are in the more developed countries and nearly 5.5 million in the less developed countries. The number of cancer cases in India is increasing due to increasing population, increase in the proportion of older people, changing patterns of lifestyles. Cancer is currently the cause of 12% of deaths worldwide. In approximately 20 years, the number of cancer deaths annually will increase from 6 million to 20 million. However, the impact of cancer is far greater than the incidence and mortality would suggest, including impact on health care costs[1].

Breast cancer in women is a major health burden. It is the most common cause of cancer deaths among women in both high resource and low resource countries[2]. Recent global cancer statistics indicate that breast cancer incidence is rising at a faster rate in populations of developing countries[2,3]. The incidence of breast cancer in Bangladesh seems to be very high. As we do not have any cancer registry with relevant data about age, sex, marital status, different types of cancers, diagnostic & treatment status and survival & mortality rate of cancer patients; it is difficult to say the exact situation in Bangladesh[4].

Prognostic factors in breast carcinoma include axillary lymph node status, tumour size, histological grade and histological subtypes[5]. Oestrogen receptor (ER) and progesterone receptor (PR) status have also been validated as factors which predict response to hormone therapy[6]. The protooncogene c-erb-2 (Her-2/neu) encodes a 185 kDa trans-membrane glycoprotein. The c-erb-2 is a member of erb family of four closely related cell surface receptors with internal tyrosine kinase domains[7]. It plays an important role in the regulation of fundamental processes such as cell growth, survival

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and differentiation and has been shown to be involved in cancer progression[8]. The aim of this study was to evaluate estrogen receptor (ER) and human epidermal growth factor receptor 2 (Her-2 /neu) status in breast carcinoma and to compare it with other prognostic parameters such as histologic type, grade, tumour size, patient age and lymph node involvement. Attempts are also taken to follow up the patients following Tamoxifen therapy.

Materials and Methods

A comprehensive study on 100 breast carcinoma cases collected from Department of Histopathology of a tertiary level hospital in Dhaka, Bangladesh was carried out between January 2010 to December 2011. These entire surgical specimens were fixed in 10% formalin, embedded in paraffin, sectioned and stained with "H&E". Before taking representative sections, size of the tumour and lymph node involvement were noted. The histological type was determined on the tissue section. Grading of the tumour was done by Scarf-Bloom-Richardson (SBR) method. Immunostaining of the ER and Her-2 was performed. Before immunostaining three to four micrometer sections were taken on sialinized slides (DAKO-S300330) then these were de-waxed in xylene, rehydrated in graded ethanol. Before going for immunostaining antigen retrieval was performed in 10 mμ citrate buffer (pH 6) putting in water bath at the temperature 95ºC to 98ºC for 40 minutes. Endogenous peroxidase activity was blocked by incubating sections in peroxidase blocking agent (DAKO-S202386). After washing in Tris Buffer saline (DAKO-S300685) ER (DAKO-M704729) and Her-2 (DAKO-A048529) were applied and slides were incubated in wet chamber at room temperature for 30 minutes. Later on DAKO Real Envision system (DAKO-K500711) was applied. DAB was used as chromogen. All slides were evaluated without knowledge of clinical outcome. For each batch of staining a known positive control slide was run for ER and Her-2 separately. ER stained slides were scored by semi quantitative scoring system established by Allred et al[9]. Her-2 was scored from 0-3 scales according to criteria set by Dako. No staining or membrane staining of <10% of tumour cells was taken as score 0. A faint membrane staining detected in >10% of tumour cells was regarded as 1+; moderate staining of membrane in

>10% of tumour cells was as 2+ and strong membrane staining in >10% tumour cells was scored as 3+. Score 3+ was considered as over expression of Her-2[10]. Though current practice requires Her-2, 2+ immunostaining to be further confirmed by Fluorescent In Situ Hybridization (FISH), which is not done in this study due to unavailability.

All the patients were managed by surgery followed by chemotherapy and hormone therapy. ER positive patients were treated by Tamoxifen and Her-2/nu overexpression cases were treated by herceptin. All the patients were followed up every 6 months up to maximum 1 and half year. The results were analyzed using the statistical software SPSS for windows, version 11. Association of clinicopathological parameters with ER and Her-2 state were analyzed using chi-square test. Statistical significance was defined as $p < 0.05$.

Results

A total 100 breast carcinoma cases diagnosed at histopathology department of a tertiary level hospital at Dhaka, Bangladesh were included in the study. All the patients were female ranged from 22 yrs to 80 yrs. Mean age was 45.64 yrs. Forty two (42%) patients were of less than 40 yrs and 58 (58%) were more than 40 yrs. Of the 100 cases included in this study 93 were diagnosed as infiltrating duct cell carcinoma, 03 were infiltrating lobular carcinoma, 02 were mucinous carcinoma, 01 medullary carcinoma and 01 was metaplastic carcinoma. The size of the tumour ranged from 1.5 cm to 10 cm with a mean size of 4.45 cm. The tumour size < 2 cm were 11, 2-5 cm were 60 (60%) and > 5 cm were 29. Among 93 infiltrating duct cell carcinoma cases, 11 (11.83%) were of grade 1, maximum that is 43 (46.24%) were of grade 2 and 39 (41.93%) were of grade 3. Among 100 cases mastectomy specimen with axillary tissue were received in 62 cases. Rests 38 were received as lumpectomy specimen. Among these 62 mastectomy cases lymph node involvement were assessed and metastatic deposits were found in 45 (72.58%) cases and 17(27.42%) were negative. Two immunohistochemical parameters ER and Her-2/neu were available for all 100 cases. For ER, quick score was '0' to '2' that was found in 62 (62%) cases and were considered as ER negative. Quick score for ER was between 3 to 8 in 38 (38%) cases and they were considered as positive (Figure-1 and 2).
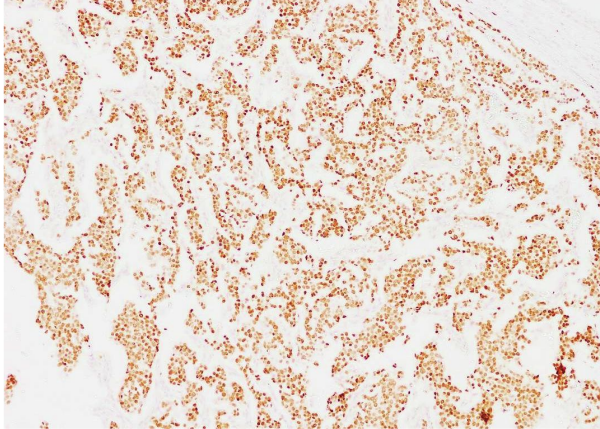
Fig-1: Immunohistochemistry for ER in ductal carcinoma, score- 6/8.
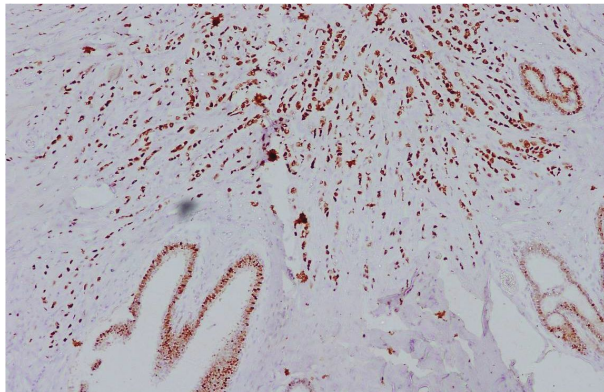
Fig-2: Immunohistochemistry for ER in lobular carcinoma, score- 7/8.

Details of clinicopathological features are described in Table-I.

Table-I: Clinicopathological Features.

| Features	Parameter	No	%
Age (n=100)	<40 yrs	42	42
	>40yrs	58	58
Tumour size (n=100)	<2 cm	11	11
	2-5 cm	60	60
	>5 cm	29	29
Histological type (n=100)	Ductal	93	93
	Non Ductal	07	07
Tumour grade (n=93)	G1	11	11.83
	G2	43	46.24
	G3	39	41.93
Lymph Node (n=62) Mastectomy with axillary tissue	Positive	45	72.58
	Negative	17	27.42
ER expression (n=100)	Negative (0-2)	62	62
	Positive (3-8)	38	38
Her-2 expression (n=100)	Negative (0-1+)	48	48
	Borderline (2+)	22	22
	Over expression (3+)	30	30

Among the ER positive 38 cases, the age range was 27 yrs to 70 yrs with a mean of 47.58 yrs, while mean age of ER negative cases was 44.51 yrs. Among 42 patients of <40 yrs, 12 (28.57%) were ER positive and 30 (71.43%) were ER negative. Among 58 patients of >40 yrs 26 (44.83%) were ER positive and 32 (55.17%) were ER negative (Table-II).

Table-II: Correlation between ER expressions with clinicopathological data.

Features	Parameter	ER +VE	ER -VE	p
Age	<40yrs (n=42)	12 (28.57%)	30 (71.43%)	<0.100
	>40yrs (n=58)	26 (44.83%)	32 (55.17%)	
Tumour size	<2 cm	05 (13.16%)	06	<0.900
	2-5 cm	22 (57.89%)	38	
	>5 cm	11 (28.95%)	18	
Histological type	Ductal	33 (35.48%)	60 (64.52%)	<0.500
	Non Ductal	05 (71.43%)	02 (28.57%)	
Tumour grade	G1 (n=11)	11 (100%)	0	<0.005
	G2 (n=44)	17 (38.64%)	27 (61.36%)	
	G3 (n=38)	05 (13.16%)	33 (86.84%)	
Lymph Node	Positive (n=45)	17 (37.78%)	28 (62.22%)	<0.900
	Negative (n=17)	6 (35.29%)	11 (64.71%)	
Her-2 expression	Negative (0-1+)	25 (65.79%)	23 (37.10%)	<0.005
	Borderline (2+)	10 (26.32%)	12 (19.35%)	
	Over expression (3+)	03 (7.89%)	27 (43.55%)	

Among ER positive cases (38), mean size of the tumour was 4.2 cm and mean size of tumours of ER negative cases was 4.6 cm. Among ER positive cases tumours size in 05 (13.16%) were <2cm, 22 (57.89%) cases were between 2 to 5cm and 11 (28.95%) were >5cm. Among 93 ductal carcinoma cases 33 (35.48%) were ER positive and 60 (64.52%) were ER negative. All the lobular and mucinous carcinoma cases were ER positive while both medullary carcinoma and metaplastic carcinoma cases were ER negative. Among the ER positive 33 ductal carcinoma cases 11 (33.33%) were of grade-1, 17 (51.52%) were of grade 2 and 05 (15.15%) were of grade-3. Among 60 ER negative cases none was of grade-1, 27 (45.0%) were of grade-2 and rest 33 (55.0%) were of grade-3. Among ER positive mastectomy specimen, axillary lymph nodes were positive in 17 cases and negative in 06 cases. While in ER negative mastectomy patient's axillary lymph nodes were positive in 28 patients and negative in 11 patients. Of 38 ER positive cases only 03(7.89%) were found with over expression of Her-2, 10(26.32%) were

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equivocal (2+) and 25 (65.79%) were negative for Her-2. Among 62 ER negative cases 27 (43.55%) were found with over expression of Her-2 (3+), 12 (19.35% were equivocal (2+) and 23 (37.10%) were negative for Her-2 (Figure-3).
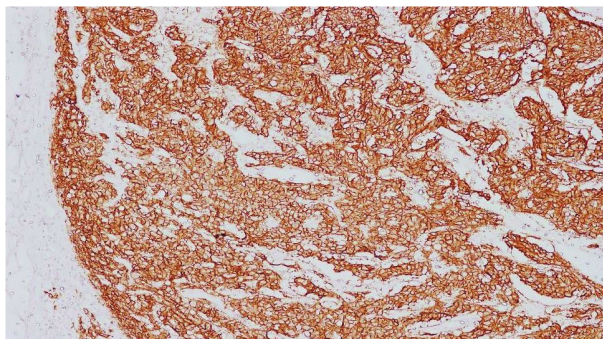
Fig-3: Immunohistochemistry for Her-2 – Score 3+, Positive.

Among the Her-2 positive (3+) cases, 30 were between 28 to 70 yrs with the mean age of 46.13 yrs, while mean age of Her-2 negative cases was 47.38 yrs. Among 42 patients of <40 yrs of age, 11 (26.19%) were Her-2 positive, 12 (28.57%) were Her-2 borderline (2+) and 19 (45.24%) were Her-2 negative (0, 1+). Among 58 patients of >40 yrs of age, 19 (32.76%) were Her-2 positive, 10 (17.24%) were Her-2 borderline (2+) and 29 (50.00%) were Her-2 negative. Among Her-2 positive 30 cases mean size in the tumour was 4.53 cm and mean size of Her-2 negative cases was 4.23 cm. Among Her-2 positive cases tumour size in 03 (10.00%) cases was <2cm, in 17 (56.67%) cases were between 2 to 5cm and in 10 (33.33%) cases >5cm. (Table-III).

Table-III: Correlation between Her-2 expressions with clinicopathological data.

Features	Parameter	Her-2 3+	Her-2 2+	Her-2 0-1+	P
Age	<40yrs	11 (26.19%)	12 (28.57%)	19 (45.24%)	<0.500
	>40yrs	19 (32.76%)	10 (17.24%)	29 (50.00%)	
Tumour size	<2 cm	03	04	04	<0.750
	2-5 cm	17	11	32	
	>5 cm	10	07	12	
Histological type	Ductal	29 (31.18%)	20 (21.51%)	44 (47.31%)	<0.750
	Non Ductal	01 (14.29%)	02 (28.57%)	04 (57.14%)	
Tumour grade	G1	01 (9.09%)	03 (27.27%)	07 (63.64%)	<0.010
	G2	09 (20.93%)	08 (18.60%)	26 (60.47%)	
	G3	19 (48.72%)	09 (23.08%)	11 (28.20%)	
Lymph Node	Positive	17 (37.78%)	9 (20.00%)	19 (42.22%)	<0.750
	Negative	6 (35.29%)	5 (29.42%)	6 (35.29%)	
ER	Negative (0-2)	27 (43.55%)	12 (19.35%)	23 (37.10%)	<0.005
	Positive (3-8)	03 (7.89%)	10 (26.32%)	25 (65.79%)	

Among 93 ductal carcinoma cases 29 (31.82%) were Her-2 positive, 20 (21.50%) were Her-2 borderline and 44 (47.31%) were Her-2 negative. One mucinous carcinoma was Her-2 positive, one lobular and medullary carcinoma were Her-2 borderline, while two lobular, one mucinous and one metaplastic carcinoma cases were Her-2 negative. Among the Her-2 positive ductal carcinoma only 01 (3.45%) case was of grade-1, 09 (31.03%) were of grade 2 and 19 (65.52%) were of grade-3. Among 20 Her-2 borderline cases 03 (15.0%) were of grade-1, 08 (40.0%) of grade-2 and 09 (45.0%) of grade-3. Among 44 Her-2 negative ductal carcinoma cases 07 (15.91%) were of grade-1, 26 (59.09%) were of grade-2 and rest 11 (25.00%) were of grade-3. Among the Her-2 positive 23 mastectomy specimens, axillary lymph nodes were positive in 17 (73.91%) cases and negative in 06 (26.09%) cases. While in Her-2 borderline 14 cases (with mastectomy) axillary lymph nodes were positive in 09 (64.29%) patients and negative in 05 (35.71%) patients. Among Her-2 negative 25 mastectomy specimen 19 (76.0%) were positive for axillary lymph nodes and 06 (24.0%) were negative of 30 Her-2 positive cases only 03 (10.00%) were found with ER positive, 27 (90.00%) were ER negative. Among 22 Her-2 equivocal cases 10(45.45%) were ER positive and 12(54.55%) were ER negative. Among 48 Her-2 negative cases, 25 (52.08%) were ER positive and 23 (47.92%) were ER negative. Her-2 borderline cases were not confirmed by her-2 FISH due to it's unavailability in Bangladesh. Among 100 cases 03 were both ER and Her-2 positive, 25 were ER positive but Her-2 negative, 10 were ER positive but Her-2 borderline, 27 were ER negative but Her-2 positive, 23 were ER negative and Her-2 negative, 12 were ER negative but Her-2 borderline.

Table-IV: ER and Her-2 state of study population (n=100).

ER+/Her-2+	ER+/Her-2-	ER+/Her-2- 2+	ER-/Her-2+	ER-/Her-2-	ER-/Her-2-2+
03	25	10	27	23	12
3%	25%	10%	27%	23%	12%

Study populations were managed following standard protocol by oncologists. After surgery all the patients were treated by chemotherapy followed by radiotherapy. After that the patients were given immunotherapy depending of their ER positivity and Her-2 expression and the patients were followed up from 6 months to maximum 2 yrs. All patients survived the follow up duration. So the overall survivality (OS) is 100%.

Discussion

Breast carcinoma is the most common malignant tumour and the leading cause of death in women with a tremendous heterogeneity in its clinical behaviour[11]. The prognosis of breast carcinoma is related to variety of clinical and pathological factors. It is well known that ER, PR and Her-2 represent the most acceptable factors for predicting prognosis, response or resistance to treatment and potential use of newer drugs[12-15]. In this study, mean age of the patients was 45.64 yrs. Most (30%) of the patients were between 31 yrs to 40 yrs age. Mudduwa LKB at Srilanka found that only 14.3% patients were of <40 yrs of age. In his study mean age was 52.5 yrs[16] which is higher than this study. In Jordan, Almasri N M[17] found median age as 47.5% and patients of <50 yrs of age was 57%. Where as Tunisia, Ayadi L et al found 51.6% patients were in <50 yrs of age group[11]. Country based study in India revealed when age between of 50-53 in breast cancer bearing women[18]. In this study, the mean size of the tumour was 4.45 cm and most of the tumours fell in 2 cm to 5 cm group. 29% tumours were of >5cm of size. Among 62 mastectomy specimens, 45 (72.58%) presented with axillary lymph node metastasis. Hence most of the tumours presented in advanced stage. Possibly it was due to lack of routine breast screening program in the country. Srilankan study revealed similar result[16]. The quick score of ER was positive that is 3 to 8 in 38% cases which are nearer to other studies carried out in neighboring countries and other Asian countries. Prevalence of 32.6% for ER positive cases was found in a study carried out in India[19]. ER and PR were found positive in 20 to 45% of Indian patients[18]. A Jordanian study revealed 50.8% ER positivity[17]. Mudduwa LKB[16] at Srilanka found 45.7% positivity for ER where as in Western countries the positivity of ER is much higher. Christopher et al[20] at United States revealed 76-78% ER positivity during 1992 to 1998. A cumulative study of 7016 case from United Kingdom revealed 73.42% ER positivity[21]. In this study, mean age of ER positive cases was 47.58 yrs and mean age of ER negative cases was 44.51 yrs. Difference is not significant. Similar results were revealed at Jordanian as well as Tunisian study[11,17]. But ER positivity was observed more in older group (>40 yrs) in comparison to younger group (<40 yrs). Rhodes et al found ER positivity more in >65 yrs of age group patients[22]. While Ayadi et al, found ER positivity more in >50 yrs of age group[11].

In this study, among ductal carcinoma cases 35.48% were ER positive. While in non ductal carcinoma ER positivity was found in 71.43% cases. Among the ductal carcinomas the ER positivity was inversely correlated with tumour grades in this study. The positivity was 100% in grade-1 while it declined to 13.16% in grade-III. In a Tunisian study ER positivity was found in 61% of duct cell carcinomas and was 22.5% in grade-III tumours[11]. Most (65.79%) of the ER positive women were Her-2/neu negative while only 7.89% showed Her-2 over expression. Most of such studies also found inverse relation between ER positivity and Her-2 over expression[11,17,23]. The study revealed overexpression (3+) of Her-2/nu in 30% cases. It is closer (34.3%) to a study carried out among Asian women in Singapore[24]. Whereas it was 17.3% in an Austrian study performed on 923 patients[25]. A Jordanian study found Her-2 overexpression in 24% cases[17]. While the Tunisian study revealed Her-2 over expression in 18.1% cases[21]. In this study variation may be due to lack of strict criteria for fixation of the tissue according to the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) guideline for Her-2 testing[10]. Mean age of women with Her-2 overexpression was 46.13 yrs. Difference between Her-2 negative women with Her-2 overexpression was not significant. While Jordanian study found significant difference in age between Her-2 overexpressing (42 yrs) and Her-2 negative (53 yrs) cases[20]. In this study, Her-2 over expression (3+) was seen in 26.19% cases of <40 yrs of age and 32.76% cases of >40 yrs of age. In Tunisian study, 21.3% women show Her-2 overexpression in <50 yrs group while it was 14.7% in >50 yrs group[11]. Some authors have suggested that Her-2 over expression is associated with young age. This study could not find significant relations between patient's age and Her-2 expression[17,25,26]. It may be due to small size of the study. Her-2 overexpressed tumours in this study were mostly (56.67%) between 2-5 cm size. Mean size of the tumour was 4.7 cm in a Jordanian study with Her-2 over expression[17]. In this study patients having Her-2 overexpression are significantly proportionate to the grade of the tumour. 48.72% of G3 duct cell carcinoma had over expressed Her-2. Taucher S, et al, found Her-2 over expression in 31.5% of G3 tumours[27]. Traina et al at Italy also found similar patterns of outcome23. In this study, Her-2 over expression was inversely correlated with ER positivity.

Among 30 overexpressed Her-2 cases only 03 (10%) were positive for ER. While Among 70 Her-2 negative cases 35 (50%) were ER positive. Sjogren S et al, Tsuda H et al, Ferrero-Pous M et al also showed inverse relation between Her-2 status and ER positivity[28,29,30]. In this study most (93%) of breast carcinomas were infiltrating ductal carcinoma. Among 93 ductal carcinoma cases 33 (35.48%) were ER positive and 60 (64.52%) were ER negative. While all the lobular and mucinous carcinoma cases were ER positive whereas both medullary and metaplastic carcinoma cases were ER negative. Among 93 ductal carcinoma cases 29 (31.82%) were Her-2 positive, 20 (21.50%) were Her-2 borderline and 44 (47.31%) were Her-2 negative. One mucinous carcinoma was Her-2 positive, one lobular and medullary carcinoma were Her-2 borderline, while two lobular, one mucinous and one metaplastic carcinoma cases were Her-2 negative. In some studies no significant relationship was observed between hormone status and histological type[31,32]. In contrast Boussen H et al suggested that Her-2 overexpression was significantly more likely in infiltrating ductal carcinoma than in infiltrating lobular carcinoma[33]. In this study, the difference in receptor status with nonductal carcinoma is probably due to very small number of non ductal carcinoma cases in the series. In this study we could follow up the cases for maximum 18 months. Over all survivality was 100%. But disease free survival could not be assessed due to lack of regular follow of the patients. In a study in Singapore General Hospital 5.2% deaths have documented among follow up of 290 breast cancer patients[24].

Conclusion
The prevalence of hormone receptor positive breast cancer is less in Asian women including women of Bangladesh which has been proved by this study too. Though the present study is a short series study but possibly the first one of this kind in this country. In contrast to western countries this study revealed more rate of Her-2/neu over expression which is similar to other studies of Asian countries. Her-2/neu overexpression is related with other poor prognostic parameters like high grade and histological type. For planning the treatment protocol of the patients' hormone receptor studies as well as Her-2/neu study are almost mandatory prerequisites.

References
1. Task Force Reports for XIth plan. National cancer control programme. Indian Council of Medical Research (ICMR), New Delhi, India. 2010, p95.

2. Bray F, MacCarron P, Parkin DM. The changing global patterns of female breast cancer incidence and mortality, Breast cancer Res 2004,6:229-39.

3. Parkin DM, Whelan SL, Ferlay J et al. Cancer Incidence in Five Continents, vol VIII. Lyon, France: IARC Press; 1997.

4. Miah MS. Knowledge, Attitude and Practice Regarding Breast Cancer Among Medical students of Bangladesh. A protocol study: Master thesis in Public health, 2006-2007. Department of Public Health & Clinical Medicine, Umea University, Umea, Sweden. May 2007.

5. Marx D, Schauer A, Reiche C et al. C-erbB2 expression in correlation to other biological parameters of breast cancer. J Cancer Res Clin Oncol 1990; 116: 15-20.

6. Yokota T, Imamura M, Teshima S et al. C-erbB-2, p53 protein expression and steroid hormone receptors in breast carcinomas: an immunohistochemical study. Anticancer Res 1999; 19: 4007-12.

7. Slamon DJ, Clark GM, Wong SG et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235: 177-82.

8. Nahta R, Esteva F. HER-2-targeted therapy: lessons learned and future directions. Clin Cancer Res 2003; 9: 5078-84.

9. Allred DC, Harvey JM, Berardo M and Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998;11: 155–68.

10. Antonio C, Wolff M, Elizabeth H et al, Guideline Summary: American Society of Clinical Oncology/College of American Pathologists Guideline. Recommendations for Human Epidermal Growth Factor Receptor Her2 Testing in Breast Cancer. Journal of Oncology Practice 2007,3;1:48-50.

11. Ayadi L, Khabir A, Amouri H et al,. Correlation of Her-2 over expression with clinicopathological parameters in Tunisian breast carcinoma. World Journal of Clinical Oncology 2008,6;112

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12. Kariya S, Ogawa Y, Nishioka A et al. Relationship between hormonal receptors, Her-2, p53 protein, bcl-2, MIB-1 status and the antitumour effects of neoadjuvant anthracycline bases chemotherapy in invasive breast cancer patients. Radiat Med 2005; 23(3) : 189-94.

13. Pinto AE, Andre S, Pareira T et al. C-erb-2 oncoprotein overexpression identifies a subgroup of estrogen receptor positive (ER+) breast cancer patients with poor prognosis. Ann Oncol 2001,12; 525-33.

14. Looi LM, Cheah PL. C-erbB-2 oncoprotein amplification in infiltrating ductal carcinoma of breast correlates to high histologic grade and loss of estrogen receptor protein. Malays J Pathol, 1998. 20:19-23.

15. Kaptain S, Tan LK, Chen B. Her-2/nu and breast cancer. Diagn Mol Pathol, 2001; 10: 139-52.

16. Muddawa LKB. Quick score of hormone receptor status of breast carcinoma; Correlation with the other clinicopathological prognostic parameters. Indian J Pathol Microbiol 2009;52:159-63.

17. Almasri NM, Hamad MA. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptor in breast carcinoma in Jordan. Breast Cancer Research 2005;7: R598-R604.

18. Agarwal G, Ramakant P. Breast cancer care in India: The current scenario and the challenges for the future. Breast Care 2008;3:21–7

19. Desai SB, Moonim MT, Gill AK, Punia RS, Naresh KN, Chinoy RF. Hormone receptor status of breast cancer in India: A study of 789 tumours. Breast 2000;9:267-70.

20. Li CI, Darling JR, Malone KE. Incidence of invasive breast cancer by hormone receptor status from 1992 to 1998. J Clin Oncol 2003;21:28-34.

21. Rhodes A, Jasani B, Balaton AJ et al. Frequency of oestrogen and progesterone receptor positivity by immunohistochemical analysis in 7016 breast carcinomas: correlation with patient age, assay sensitivity, threshold value and mammographic screening. J Clin Pathol 2000; 53:688-96.

22. Rhodes A, Jasani B, Balaton A et al carried out a study. Frequency of oestrogen and progesterone receptor positivity by immunohistochemical analysis in 7016 breast carcinomas: correlation with patient age, assay sensitivity, threshold value and mammographic screening. J Clin Pathol 2000;53:688-96.

23. Traina A, Agostara B, Marasa L et al. HER2/neu Expression in Relation to Clinicopathologic Features of Breast Cancer Patient. Ann N.Y.Acad.Sci.2006;1089: 159-67.

24. Sathiyamoorthy S, Yan WK, Siong KK et al. Overexpression of c-erb-2 correlates with nuclear morphometry and prognosis in breast carcinoma in Asian Women. Pathology 2006;38(6):528-33.

25. Huang HJ, Neven P, Drijkoningen M et al. Hormone receptors do not predict the Her-2/neu status in all age women with an operable breast cancer. Ann Oncol 2005;16:1755-61.

26. Seo BK, Pisano ED, Kusimak CM et al. Correlation of Her-2/neu over expression with mammography and age distribution in primary breast carcinomas. Acad Radiol 2006;13:1211-18.

27. Taucher S, Rudas M, Mades R et al. Do We Need HER-2/neu Testing for All Patients with Primary Breast Carcinoma? Cancer 2003;98:2547–53.

28. Sjogren S, Inganas M, Lindgren A et al. Prognostic and predictive value of c-erbB-2 over expression in primary breast cancer, alone and in combination with other prognostic markers. J Clin Oncol 1998;16:462-9.

29. Tsuda H, Iwaya K, Fukutomi T and Hirohashi S. p53 mutations and c-erbB-2 amplification in intraductal and invasive breast carcinomas of high histologic grade. Jpn J Cancer Res 1993; 84:394-401.

30. Ferrero-Pous M, Hacene K, Bouchet C et al. Relationship between c-erbB-2 and other tumour characteristics in breast cancer prognosis. Clin Cancer Res 2000;6:4745-54.

31. Bamberger AM, Milde-Langos K, Schulte HM and Loning T. Progesterone receptor isoforms PR-B and PR-A, in breast cancer: correlations with clinicopathologic tumour parameters and expression of AP-1 factors. Horm Res 2000;54:32-7.

32. Killing N, Yaldiz M. p53, c-erbB-2 expression and steroid hormone receptors in breast carcinoma: correlations with histomorphological parameters. Eur Gynaecol Oncol 2004;25:606-10.

33. Boussen H, Bouzaiene H, Ben Hassouna J, Gamoudi A, Benna F, Rahal K. Inflammatory breast cancer in Tunisia: Reassessment of incidence and clinicopathological features. Semin Oncol 2008;35(1):17-24.