Original Article

A Study of Hormone Receptor Status in Breast Carcinoma and use of HER2-Targeted Therapy in a Tertiary Care Center of India

Abstract

Aims: The aim is to study the hormone receptor status, association of HER2 expression with prognostic factors and use of HER2-targeted therapy in North Indian breast cancer patients. Subjects and Methods: Immunohistochemistry reports of 288 breast cancer patients registered in the department of Radiotherapy, SMS Medical College, Jaipur in 2015-2016 were analyzed for estrogen receptor (ER), progesterone receptor (PR), and c-erb B-2 protein (HER2/neu) expression. Equivocal HER2 (2+) was further confirmed by fluorescent in situ hybridization (FISH). Number of patients receiving HER2/neu-targeted therapy was also studied. Results: For ER, positive status was more common (56%), whereas for PR and HER2/neu, negative status was more common (59% and 60% resp.). HER2 status was unknown for 25% patients. The percentage of equivocal HER2 (immunohistochemistry 2+) cases showing amplification on FISH was also high (56.7%). The percentage of eligible cases for targeted therapy actually receiving it was low (28%). The percentage of triple negative phenotype (ER-/PR-/HER2-) was high (29.8%). Triple-negative breast cancer phenotype was more common in young-aged premenopausal women but was not statistically significant. All HER2/neu + cases were infiltrating ductal carcinoma. HER2/neu expression was significantly higher with large tumor size (P = 0.001), high tumor grade (P < 0.001), advanced stage (P = 0.001), greater number of positive lymph nodes (P = 0.02), and ER/PR negativity (P < 0.001). Conclusions: Most of the breast cancer patients are ER and/or PR positive and HER2/neu negative. The percentage of triple-negative phenotype is higher. More than half of HER2/neu 2+ cases show amplification on FISH assay. The percentage of eligible patients actually receiving targeted therapy is low. HER2/neu protein expression is significantly higher with adverse features such as large tumor size, high grade, advanced stage, greater number of positive lymph nodes, and ER/PR negativity.

Keywords: *C-erb B-2 protein, estrogen receptor, fluorescent in situ hybridization, progesterone receptor, targeted therapy, triple-negative breast cancer*

Introduction

Breast cancer is the most common cancer diagnosed in the women not only worldwide but also in India.^[1] Breast cancer patients have good overall survival when treated with combination of surgery, chemotherapy, targeted radiotherapy, therapy, and hormonal therapy as per the indication.^[2] Estrogen receptor and progesterone receptor (ER and PR) and c-erb B-2 protein (HER2/neu) status remain one of the most important factors in determining response to treatment and prognosis of disease. The use of various hormonal agents such selective ER modulators tamoxifen as aromatase inhibitors anastrozole and and letrozole depends exclusively on the expression of ER/PR,[3] whereas the use of recently developed novel-targeted

agents such as trastuzumab, pertuzumab, and lapatinib depends exclusively on the expression of HER2/neu receptors.^[4,5]

Subjects and Methods

The present study was carried out on female breast cancer patients registered in the department of Radiotherapy, SMS Medical College and Hospital, Jaipur; during year 2015–2016 with histopathologically proven diagnosis of invasive breast cancer and a pathology review available from our institute. A total of 288 patients were found eligible. Various patients', tumor- nd treatment-related parameters were recorded after obtaining consent from the patient. The biopsy was analyzed immunohistochemically for ER, PR, and HER2/neu expression. Equivocal HER2 (2+) was further confirmed by fluorescent in situ hybridization (FISH) assay. Number of patients who actually

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received HER2/neu-targeted therapy (trastuzumab and/ or lapatinib) among eligible ones was also studied. The biopsy sample analyzed for hormone receptor status was the initial one, before any chemotherapy, targeted therapy, definitive surgery, radiotherapy, or hormonal therapy. For the new cases reporting in the department, biopsy was taken and sample was analyzed at our institute. For the postoperated-referred patients with the history of neoadjuvant chemotherapy, the prechemo biopsy sample was analyzed at institute and such patients were included only if the material was found to be adequate at least for ER/PR analyses. Cases without initial prechemo sample or with inadequate sample for proper review were excluded from the study. All those patients with equivocal HER2/neu (2+ on immunohistochemistry [IHC]) who denied for FISH analysis were also excluded from the study.

All cases were immunohistochemically evaluated for ER, PR, and HER2/neu expression using standard immunoperoxidase method. The tests were interpreted with internal controls. Immunostaining was carried out on thin sections of formalin-fixed, paraffin-embedded tissue with fixation within 1 h in 10% neutral buffered formalin for at least 6 h and no longer than 72 h. ER and PR were scored as per Allred score which is a semi-quantitative system that takes into consideration the proportion of positive cells (Proportion score - 0 for none positive cell, 1 for 1%, 2 for 1%-10%, 3 for 10%-33%, 4 for 33%-66%, and 5 for 66%-100% positive cells) and staining intensity (intensity score -0 for no stain, 1 for weak, 2 for intermediate, and 3 for strong staining). The two scores were then summed to produce total scores of 0 through 8. A score of 0-2 was regarded as negative, while 3-8 as positive.^[6,7] HER2/neu scoring of IHC slides was done as per the recommended American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines 2013. Score 0 and 1 were interpreted as negative, score 2 as equivocal, and score 3 as positive.^[8]

For equivocal HER2/neu, cases were referred for the FISH assay in the referral laboratories as the facility for FISH was not available in our institute. The fields containing invasive tumor component with nonoverlapping tumor nuclei were chosen for interpretation for FISH. The total number of red and green signals counted in the tumor nuclei was recorded. The ratio of the HER2 (red) to chromosome enumeration probe (CEP) 17 (green) signals for at least 20 cells was calculated. The cutoff point for HER2/neu amplification was a HER2/CEP 17 ratio of \geq 2.0, with an average HER2 copy number of \geq 4.0 signals/ cell. Appropriate positive and negative controls were run with the test samples in the referral laboratories. The interpretation of the FISH assay was done following the ASCO/CAP guidelines recommendation 2013.^[8,9]

Statistical analysis

For statistical analysis, all data were recorded and analyzed on Microsoft Excel 2007 and XLSTAT software

version 2017 for Windows (Addinsoft, New York, USA). Chi-square test was used for categorical data. The P value reports were two-tailed and an alpha level of 0.05 was used to assess statistical significance.

Results

Baseline patient and tumor characteristics are shown in Table 1 and Figure 1. Most of the patients were older than 40 years, postmenopausal belonging to the urban background. Most of the tumors were right sided, situated in upper outer quadrant, infiltrating ductal carcinoma (IDC), not otherwise specified on histopathological examination (HPE), Grade III, and American Joint Committee on Cancer (AJCC) stage IIB.

Receptor expression pattern. distribution of HER2/neu expression and the use of HER2-targeted therapy is shown in Table 2. The present study favored ER positive (56% vs. 44%), PR negative (59% vs. 41%), and HER2/neu negative (60% vs. 15%) status in invasive breast cancer. In the present study, HER2/neu status was unknown in about a quarter of patients. This percentage was quite large and was because of either the lack of tissue or poor preservation of the specimen. The equivocal HER2/neu, that is, 2+ on IHC, was further tested by FISH assay. More than half of equivocal HER2/neu cases showed amplification on FISH. The eligible cases for targeted therapy were those with either HER2/neu 3+ on IHC or HER2/neu 2+ with positive FISH test. Only one-fourth of eligible cases for targeted therapy actually received it.

The percentage of triple-negative phenotype (ER-/PR-/HER2-) was higher as compared to the Western world (29.8%). ER and/or PR expression increased and triple-negative phenotype decreased with increasing age, but this was not found to be statistically significant in our study [Table 1].

Table 3 and Figure 2 shows association of HER2/ neu expression with various prognostic factors. HER2/ neu expression was not significantly associated with age (P = 0.87), menopausal status (P = 0.86), and site of the tumor (P = 0.76). All HER2/neu+ cases in our study were IDC on HPE, none other phenotype expressed HER2 positivity. HER2/neu protein overexpression was significantly higher with large tumor size (P = 0.001), high tumor grade (P < 0.001), advanced stage (P = 0.01), greater number of positive lymph nodes (P = 0.02), and ER and/or PR negative status (P < 0.001).

Discussion

In the present study, most of the tumors were ER + (n = 161, 56%), PR - (n = 170, 59%), and HER2/neu - (n = 173, 60%). Therefore, endocrine-responsive tumors, that is, those expressing at least one among ER or PR (ER and/or PR+) comprised 57.3% (n = 165) of all invasive carcinomas. This is in agreement with what has been reported in the literature. In a study among 2001 Indian patients, Ghosh *et al.* have reported hormone responsive

Table 1: Patient and tumour characteristics of overallbreast cancer and triple negative breast cancer					
	Cohort, <i>n</i> (%)	n (%)			
Age (years)					
≤40	84 (29)	32 (36.3)	0.20		
>40	204 (71)	56 (63.7)			
Geographic distribution					
Rural	115 (40)	45 (51)	0.29		
Urban	173 (60)	43 (49)			
Menopausal status					
Pre	120 (41.7)	49 (55.6)	0.02		
Post	168 (58.3)	39 (44.4)			
Site					
Left	135 (47)	32 (36.3)	0.08		
Right	153 (53)	56 (63.7)			
Quadrant					
UOQ	124 (43)	47 (53.6)	0.16		
UIQ	37 (13)	15 (17)			
Central	78 (27)	15 (17)			
LOQ	29 (10)	8 (9)			
LIQ	20 (7)	3 (3.4)			
HPE					
IDC	278 (96.7)	88 (100)	0.37		
ILC	6 (2)	0			
Mucinous	3 (1)	0			
Others	1 (0.3)	0			
Grade					
Ι	14 (5)	5 (5.6)	0.37		
II	112 (39)	27 (30.7)			
III	162 (56)	56 (63.7)			
AJCC stage					
Ι	0	0	0.12		
IIA	80 (28)	21 (23.8)			
IIB	86 (30)	25 (28.5)			
IIIA	66 (23)	19 (21.7)			
IIIB	6 (2)	1 (1.1)			
IIIC	35 (12)	10 (11.3)			
IV	15 (5)	12 (13.6)			
T stage					
T1	17 (6)	4 (4.5)	0.004		
Т2	150 (52)	64 (72.9)			
Т3	89 (31)	12 (13.6)			
Τ4	32 (11)	8 (9)			
N stage					
NO	80 (28)	36 (41)	0.12		
N1	104 (36)	26 (29.5)			
N2	64 (22)	18 (20.5)			
N3	40 (14)	8 (9)			

AJCC-American Joint Committee on Cancer; HPE-Histopathological Examination; IDC – Infiltrating Ductal Carcinoma; ILC – Infiltrating Lobular Carcinoma; LIQ – Lower Inner Quadrant; LOQ – Lower Outer Quadrant; N – Nodal; T – Tumor; TNBC – Triple Negative Breast Cancer; UIQ – Upper Inner Quadrant; UOQ – Upper Outer Quadrant

phenotype in 51.2% patients.^[10] In a study conducted by Chen *et al.*, hormone responsive phenotype was seen in

Table 2: Overall receptor expression pattern in entire
cohort, Distribution of Her2/neu expression and the use
of Her2 targeted therapy

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Parameters	Number (%)
Subtype Classification	
Hormone Responsive (ER &/or PR +)	165 (57.3)
Her2/neu +	43 (15)
TNBC (ER-PR-Her2-)	88 (30.5)
Her2/neu expression	
Negative	173 (60)
IHC 0	98
IHC 1+	62
IHC 2+, FISH -	13
Positive	43 (15)
IHC 2+, FISH +	17
IHC 3+	26
Unknown	72
Targeted therapy	
Eligible patients	43
Therapy taken	12 (28)
Not taken	31 (72)
Source of finance	
Self-finance	9 (75)
Insurance	3 (25)

+-Positive; --Negative; ER-Estrogen Receptor; FISH-Fluorescent In Situ Hybridization; IHC - Immunohistochemistry; PR - Progesterone Receptor; TNBC - Triple Negative Breast Cancer

75% of the patients, but this study was conducted on only 64 patients who expressed HER2/neu.^[11] Ahmed *et al.* studied hormone expression in 137 Yemeni women and found expression of ER, PR, HER2/neu in 43.8%, 27%, and 30.6% patients, respectively.^[12] Faheem *et al.* studied hormone expression in 1226 Pakistani women and found ER, PR, and HER2/neu positivity in 763 (62.2%), 738 (60.1%), and 478 (38.9%) patients, respectively.^[13]

In the present study, HER2/neu expression was present in 15% (n = 43) patients. This is slightly lower than 22% reported by Ghosh *et al.* in 2001 Indian patients,^[10] 30.6% by Ahmed *et al.* in 137 Yemeni women,^[12] and 38.9% by Faheem *et al.* in 1226 Pakistani women.^[13] This may be explained by the fact that in the present study, HER2/neu expression was unknown in 72 (25%) patients. This was quite high and was mainly because many of the tumor blocks referred from outside were poorly preserved or had insufficient tissue for review.

Equivocal HER2/neu (HER2 2+ on IHC) was seen in 30 (10.4%) patients. These were the candidates for FISH assay. On applying FISH, out of these thirty patients, 17 (56.7%) turned out to be positive, so the final number of HER2/neu + patients was 43, that is, 15% (26 patients with HER2/neu 3+ expression on IHC and 17 patients with HER2/neu 2+ on IHC with HER2 amplification by FISH). Ghosh *et al.* have also reported similar finding in their report, HER2/neu scoring was negative, that is, 0/1

characteristic	s with Her2/nd	atient and tur	lour
Reseline	Her2/neu +	Her2/neu -	P
characteristics	(n, %)	(n, %)	1
Age (vears)	((,,,,,))	((,,,,,))	
<40	14 (20.5)	54 (79.5)	0.87
>40	29 (19.6)	119 (80.5)	
Menopausal status	_, (_,,)		
Pre	17 (19.4)	71 (80.6)	0.86
Post	26 (20.4)	102 (79.6)	
Site	_ ()		
Left	19 (19)	81 (81)	0.76
Right	24 (20.7)	92 (79.3)	
Histopathology	2. (20.7))_()))	
IDC	43 (29)	163 979)	0.46
ILC	0	6 (100)	
Mucinous	0	3 (100)	
Others	0	1 (100)	
Grade		- ()	
I	1 (8.3)	11 (91.7)	< 0.001
I	5 (5.9)	80 (94.1)	0.001
III	85 (31)	82 (69)	
Tumour size (cm)			
<2	1 (9)	10 (91)	0.001
2-5	17 (14.4)	101 (85.6)	
>5	14 (21.5)	51 (78.5)	
T4 lesion	11 (50)	11 (30)	
Lymph nodes involved			
0	10(16.7)	50 (83.4)	0.08
1-3	13 (17.8)	60 (82.2)	
4-9	7 (13.7)	44 (86.3)	
>10	13 (40.6)	19 (59.4)	
AJCC stage			
IIA	9 (13.6)	57 (86.4)	0.01
IIB	11 (15.7)	59 (84.3)	
IIIA	8 (18.6)	35 (81.4)	
IIIB	1 (25)	3 (75)	
IIIC	7 (31.8)	15 (68.2)	
IV	7 (63.7)	4 (36.3)	
Estrogen Receptor	()		
+	5 (5.5)	86 (94.5)	< 0.001
-	38 (30.4)	87 (69.6)	
Progesterone Receptor			
+	4 (6.5)	57 (93.5)	0.002
-	39 (25.1)	116 (74.9)	
Hormone Responsive		- ()	
Either ER or PR+	7 (7.5)	87 (92.5)	< 0.001
Both ER & PR-	36 (29.5)	86 (70.5)	

+ – Positive; - – Negative; AJCC – American Joint Committee on Cancer; ER – Estrogen Receptor; IDC – Infiltrating Ductal Carcinoma; ILC – Infiltrating Lobular Carcinoma; PR – Progesterone Receptor

in 1424 (71.2%), 2+ in 163 (8.1%), and 3+ in 335 (16.7%) patients. 65.3% of HER2/neu 2+ showed amplification on FISH assay.^[10] This mandates the implication of FISH test for equivocal HER2/neu patients.

The percentage of triple-negative breast cancer (TNBC) defined as lack of expression of all three receptors, was higher in the present study as compared with the Western world (n = 88, 30.5%) but was consistent with most other reports from the nation. Ghosh et al. have reported TNBC phenotype in 29.8% patients.^[10] On subgroup analysis in the present study, on comparing with overall cohort of breast patients, TNBC was found to be significantly higher in premenopausal women (P = 0.02) and T2 tumors (P = 0.004), this is consistent with the findings of Ghosh *et al.*^[10] The rate of hormone receptor positivity, that is, ER- and/or PR-positive irrespective of HER2 status was higher in older age group, whereas TNBC was seen more common in younger age group. Although ER and/ or PR expression increased and triple-negative phenotype decreased with increasing age, but this was not found to be statistically significant in the present study.

A separate analysis was done for HER2/neu over expression in breast tumor. On initial analysis, Her2/neu status was negative (IHC 0 or 1+) in 55.6% (n = 160), equivocal (IHC 2+) in 10.4% (n = 30), and positive (IHC 3+) in 9% (n = 26) patients. On applying FISH assay to equivocal HER2/neu, 17 out of 30 (56.7%) showed HER2 amplification. Hence, the final status of HER2/neu expression was unknown in 25% (n = 72), negative in 60% (n = 173; 160 HER2 0 or 1+ on IHC and 13 IHC 2+ with negative FISH) and positive in 15% (n = 43; 26 IHC 3+ and 17 IHC 2+ with positive FISH) patients.

HER2/neu expression was exclusively seen in IDC in the present study. No other tumor subtype expressed HER2/ neu overexpression. This was because of very less number of other histopathology phenotypes (n = 10, 3.4%). In literature, there have been studies with more number of lobular carcinoma. Hoff *et al.* in a study over 401 women found lobular carcinomas less likely to have HER2/ neu amplification than ductal carcinomas.^[14] HER2/neu amplification was higher in high grade and metastatic tumors. In another study, Rosenthal found HER2/neu amplification more in ductal (48%) than lobular (13%) carcinoma (P < 0.001).^[15] This is consistent with our result.

As far as grade of the tumor is concerned, HER2/neu overexpression was seen in 1/12 (8.3%) Grade I, 5/85 (5.9%) Grade II, and 37/119 (31%) Grade III tumors. This association was statistically significant for higher tumor grade (P < 0.001). With respect to the tumor size, HER2/neu overexpression was seen in 1/11 (9%) T1 lesions, 17/118 (14.4%) T2 lesions, 14/65 (21.5%) T3 lesions, and 11/22 (50%) T4 lesions. This association was statistically significant for larger tumor size (P = 0.008). With respect to the number of positive lymph nodes, HER2/neu overexpression was seen in 10/60 (16.7%) N0 lesions, 13/73 (17.8) N1 lesions, 7/51 (13.7%) N2 lesions, and 13/32 (40.6%) N3 lesions. This association was also statistically significant (P = 0.02). With respect



Figure 1: Association of overall breast cancer and triple negative breast cancer with various patient and tumour related factors



Figure 2: Association of Her2/neu expression with various patient and tumour related factors

to AJCC stage of the tumor, HER2/neu overexpression was seen in 9/66 (13.6%) Stage IIA, 11/70 (15.7%) Stage IIB, 8/43 (18.6%) Stage IIIA, 1/4 (25%) Stage IIIB, 7/22 (31.8%) Stage IIIC, and 7/11 (63.7%) stage IV. This association again was statistically significant with higher AJCC stage (P = 0.01). It can be inferred that HER2/neu

expression increased with increasing size and stage of the tumor. Almasri and Hamad studied association of expression of HER2/neu with various prognostic parameters over 91 Jordanian breast cancer women and showed that HER2/ neu expression is inversely associated with age (P < 0.001 favoring young age) and directly related to large tumor

size (P = 0.13) and node positivity (P = 0.29), but this did not reach statistical significance as nodal status was unknown in 50% of their patients.^[16] Faheem *et al.* studied hormone expression in 1226 Pakistani women and found significant association between HER2/neu overexpression with premenopausal status (P < 0.001), large tumor size (P < 0.001), involvement of skin (P < 0.001) and lymph nodes (P < 0.001), and presence of distant metastases (P < 0.001).^[13] However, no significant association was detected between ER, PR, HER2/neu and disease recurrence.

The HER2/neu expression was found to be inversely associated with expression of ER (38/125 [30.4%] ER-patients, P < 0.001) and PR (39/155 [25.1%] PR-patients, P = 0.002). Thus, association of HER2/neu expression with endocrine-responsive tumor, that is, expressing either ER or PR was inversely related (36/122 [29.5%] ER-PR-patients, P < 0.001). However, no significant association was observed between HER2/neu expression and age of the patients (P = 0.87), menopausal status (P = 0.86), and site of the tumor (P = 0.76). Faheem *et al.* found an inverse relationship between hormonal receptors expression and HER2/neu expression.^[13] Almasri and Hamad also showed that HER2/neu expression (82% HER2+ patients were lacking ER and PR expression).^[16]

The eligible cases for targeted therapy were those with either HER2/neu 3+ on IHC (n = 26) or HER2/neu 2+ on IHC with HER2 amplification on FISH assay (n = 17). Out of these 43 eligible patients, only 12 (28%) actually opted for it. This was largely because of higher cost of the therapy and its noninclusion in various government aided social schemes for poor patients prevailing in the state at the time of writing this article, so most of the patients were unable to bear the cost of the treatment. Out of the 12 patients, 3 got the medicine from pensioners fund and the remaining nine patients self-financed for it. Ghosh et al. have reported that out of 441 (22%) eligible patients, only 38 (8.6%) patients actually received HER2-targeted therapy. Out of 38 patients, 20 (52.6%) received it as a part of ongoing trial, 13 (34.2%) self-financed it, and remaining 5 (13.2%) were covered under some insurance scheme.^[10] Thus, the percentage of eligible cases for targeted therapy actually receiving it is quite low (28%) in the present study.

Conclusion

Most of the breast cancer patients are hormone responsive, that is, ER and/or PR positive and HER2/neu negative. The percentage of triple-negative phenotype is high. ER and/or PR expression increased and triple-negative phenotype decreased with increasing age, but this was not found to be statistically significant in the present study. HER2/neu status remains unknown for about a quarter of the patients, largely because of poor preservation of sample obtained at the periphery. More than half of the equivocal HER2/neu on IHC shows HER2 amplification on FISH analysis mandating FISH assay to be done for all HER2/neu equivocal cases. HER2/neu protein overexpression is significantly higher with adverse features such as large tumor size, high grade, advanced stage, greater number of positive lymph nodes, and negative ER/PR status; but not with age, menopausal status, and site of the tumor. The percentage of eligible patients actually receiving targeted therapy remains low.

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Conflicts of interest

There are no conflicts of interest.

References

- National Cancer Registry Programme, Indian Council of Medical Research, Three Year Report of Population Based Cancer Registries 2012-2014, Incidence, Distribution, Trends in Incidence Rates and Projections of Burden of Cancer, Bengaluru, India; 2016. Ch. 2; 2016. p. 9-26. Available from: http://www.ncrpindia.org/ALL_NCRP_REPORTS/PBCR_ REPORT_2012_2014/ALL_CONTENT/PDF_Printed_Version/ Chapter 2_Printed.pdf. [Last accessed on 2017 Feb 02].
- NCCN Clinical Practice Guidelines in Oncology: Breast Cancer, Version 2.2017. National Comprehensive Cancer Network. Available from: http://www.nccn.org/professionals/physician_gls/ pdf/breast.pdf. [Last accessed on 2017 May 10].
- 3. Tamoxifen for early breast cancer: An overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 1998;351:1451-67.
- McKeage K, Perry CM. Trastuzumab: A review of its use in the treatment of metastatic breast cancer overexpressing HER2. Drugs 2002;62:209-43.
- Smith I, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, *et al.* HERA study team 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: A randomised controlled trial. Lancet 2007;369:29-36.
- Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998;11:155-68.
- Allred DC, Bustamante MA, Daniel CO, Gaskill HV, Cruz AB Jr. Immunocytochemical analysis of estrogen receptors in human breast carcinomas. Evaluation of 130 cases and review of the literature regarding concordance with biochemical assay and clinical relevance. Arch Surg 1990;125:107-13.
- Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, *et al.* Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Arch Pathol Lab Med 2014;138:241-56.
- 9. Panjwani P, Epari S, Karpate A, Shirsat H, Rajsekharan P, Basak R, *et al.* Assessment of HER-2/neu status in breast cancer using fluorescence *in situ* hybridization & immunohistochemistry:

Experience of a tertiary cancer referral centre in India. Indian J Med Res 2010;132:287-94.

- Ghosh J, Gupta S, Desai S, Shet T, Radhakrishnan S, Suryavanshi P, *et al.* Estrogen, progesterone and HER2 receptor expression in breast tumors of patients, and their usage of HER2-targeted therapy, in a tertiary care centre in India. Indian J Cancer 2011;48:391-6.
- Chen W, He J, Song S, Wang M, Wu H, Wang X, et al. Efficacy of TCH/TEC neoadjuvant chemotherapy for the treatment of HER-2-overexpressing breast cancer. Oncol Lett 2015;9:1922-6.
- Ahmed HG, Al-Adhraei MA, Al-Thobhani AK. Correlations of hormone receptors (ER and PR), Her2/neu and p53 expression in breast ductal carcinoma among Yemeni women. Open Cancer Immunol J 2011;4:1-9.
- 13. Faheem M, Mahmood H, Khurram M, Qasim U, Irfan J.

Estrogen receptor, progesterone receptor, and her 2 neu positivity and its association with tumour characteristics and menopausal status in a breast cancer cohort from northern Pakistan. Ecancermedicalscience 2012;6:283.

- Hoff ER, Tubbs RR, Myles JL, Procop GW. HER2/neu amplification in breast cancer: Stratification by tumor type and grade. Am J Clin Pathol 2002;117:916-21.
- 15. Rosenthal SI, Depowski PL, Sheehan CE, Ross JS. Comparison of HER-2/neu oncogene amplification detected by fluorescence *in situ* hybridization in lobular and ductal breast cancer. Appl Immunohistochem Mol Morphol 2002;10:40-6.
- Almasri NM, Al Hamad M. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. Breast Cancer Res 2005;7:R598-604.