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Comparison of HER-2/neu with histological grade and hormone (ER, PR) status in carcinoma breast

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Abstract--Objectives:- The study was performed with the aim to compare HER-2/neu over expression with age, size of tumor, histopathological type, grade of tumor, lymph node positivity, NPI, and hormone receptors ER and PR. Methods :- The study was conducted on 66 cases of breast carcinoma. All the cases underwent immunohistochemistry for ER, PR and HER-2/neu over expression. HER-2/neu over expression was compared with prognostic factors. Results:- The age range of these patients was from 30-70 years with mean age of 49.92 years. HER-2/neu was strongly positive (score 3) in 12/66 (18.18%), moderate or equivocal (score 2) in 7 /66 (10.60 %) and mild (score 1) in 16/66 (24.24%) and it was negative (score 0) in 31/66 (46.97 %). Estrogen Receptor (ER) was positive in 38/66 (57.57%) cases, progesterone receptors (PR) was positive in 30/66 (45.45%) cases. Triple negative cases were 16/66 (24.24%) and triple receptor positive cases were 04/66 (6.06%). On comparison of HER-2/neu over expression with various prognostic factors it was observed that there was no statistically significant association of HER-2/neu positivity with size, morphological type, grade, NPI ($p > 0.05$) whereas statistically significant association was seen in patients more than 45

years of age ($p = 0.035$) and in lymph node positive cases ($p = 0.01$)
Conclusions:- It is concluded from the study that HER-2/neu is directly related to age and lymph node status of the patients

Keywords---HER-2/neu, estrogen receptors, progesterone receptors, carcinoma breast, NPI.

Introduction

Breast Cancer is the most common malignancy in women, affecting one in eight in the western World.⁽¹⁾ In India Breast cancer is the second most common cancer in women after cervical cancer. However, recent studies have indicated a changing trend with an increasing incidence of breast cancer and a decreasing incidence of cervical cancer.⁽²⁾ The rising incidence of breast cancer is attributed to westernization of the country. The age adjusted incidence rate (AAR) of breast cancer in India varied from 6.8 to 33 per 100,000 women as per the annual report of the National Cancer Registry Programme under the ICMR.⁽³⁾ The tumor is highly heterogeneous, with wide range of biological, pathological and clinical characteristics. Many factors have been implicated in the prognosis of breast cancer. Age of the patient, menopausal status, family history, tumor size, grade, lymph node status and distant metastasis are among the numerous factors implicated. At the molecular level these include ER, PR, HER-2/neu, Cyclin D1 and VEGF among others. The presence of ER/PR has been associated with better prognosis while HER-2/neu amplification with a worse outcome, more so in node positive patients.⁽⁴⁾

Prognostic and predictive factors are used in the management of breast cancer. ER, PR, HER-2/neu are prognostic as well as predictive factors.⁽⁵⁾ The clinical importance of HER2/neu diagnosis has increased even more with the advent of new anti cancer drug trastuzumab, a humanized monoclonal antibody to the extra cellular domain of HER-2/neu oncoprotein, which has greatly improved the prognosis.⁽⁶⁾ Some breast tumors are single hormone receptor positive (ER positive/ PR negative or ER negative/ PR positive) and biologically and clinically different from double hormonal receptor positive (ER positive/ PR positive) and double hormonal receptor negative (ER negative/ PR negative) subtypes.^(7,8) Compared to hormonal receptor negative breast cancers, hormonal receptor positive breast cancers have less aggressive clinicopathological features and have a better prognosis since they benefit from endocrine treatment. The study was performed with the aim to compare HER-2/ neu over expression with some clinical prognostic factors, histopathological grade and ER/PR status.

Material and Methods

The study was conducted on 66 confirmed cases of invasive breast carcinoma (44 modified radical mastectomy, 22 lumpectomy) observed during a period of last 5 years (July 2011 to June 2016) after taking institutional ethical clearance.

Inclusion criteria- All confirmed cases of carcinoma breast were included in the study.

Exclusion criteria- (i). Non availability of representative tissue of the tumors.

(ii). Poor tissue processing.

(iii). Tru cut biopsy specimen.

The age, side, site, size, histological type, grade, lymph node status (except in lumpectomy cases), stage (pT) were studied in all the cases. Nottingham Prognostic Index (NPI) was calculated in cases where lymph node status was available. Sections from these cases were taken and IHC was put for ER, PR, HER-2/neu.

Immunohistochemistry (IHC) was performed using-

- Primary antibodies for HER-2/neu, a monoclonal mouse anti HER-2/neu clone SP 3- prediluted liquid mouse monoclonal antibody (genova scientific).
- Primary antibodies for ER- Genova scientific prediluted monoclonal rabbit anti ER protein/ clone-SP1.
- Primary antibodies for PR- Genova scientific prediluted monoclonal mouse anti PR protein/ clone-SP2

Positive and negative controls were run with every batch of the IHC.

Scoring was done according to CAP guidelines, 2013.⁽⁹⁾

HER-2/neu status was compared with : Age, Size of tumor, Histological type, Grade of tumor⁽¹⁰⁾, Lymph node positivity, Nottingham Prognostic Index ⁽¹¹⁾, ER/PR status

Statistical Analysis

It was done by -Descriptive statistics and chi square test. The values were considered significant when p value was <0.05.

Results

The age range of these patients was from 30-70 years with mean age of 49.92 years.

HER-2/neu positivity –

Strong positivity (score 3) = in 12/66, 18.18%

Moderate or equivocal (score 2) = in 7/66, 10.60%

Mild (score 1) = in 16/66, 24.24%

Table (1)-HER-2/neu status according to age (≤ 45 & >45) (n=66)

Age in years	Total number of cases	HER-2/neu							
		Negative				Equivocal		Positive	
		Score 0		Score 1+		Score 2+		Score 3+	
		n	%	n	%	n	%	n	%
≤ 45	27	16	59.25	6	22.22	4	14.81	1	3.70
> 45	39	15	38.46	10	25.64	3	7.69	11	28.20

(p=0.035)

Table 2- Her-2/neu status according to size (n=66)

Size	Total number of cases	HER-2/neu							
		Negative				Equivocal		Positive	
		Score 0		Score 1+		Score 2+		Score 3+	
		n	%	n	%	n	%	n	%
≤ 2 cm	8	4	50.00	1	12.50	1	12.5	2	25.00
$>2-5$ cm	36	17	47.22	8	22.22	6	16.67	5	13.89
>5 cm	22	11	50.00	6	27.27	0	0	5	22.72

(p>0.05)

Size of the tumor varied from 1.6 cm to 17.0 cm and majority were between 2 to 5 cm.

TABLE 3- HER-2/neu status according to histological type (n=66)

Histological Type	Total number of cases	HER-2/neu							
		Negative				Equivocal		Positive	
		Score 0		Score 1+		Score 2+		Score 3+	
		n	%	n	%	n	%	n	%
Invasive Carcinoma of No Special Type	60	27	45	15	25	6	10	12	20
Invasive Lobular carcinoma	02	2	100	0	0	0	0	0	0
Carcinoma with neuroendocrine differentiation	02	1	50	1	50	0	0	0	0
Mucinous carcinoma	01	1	100	0	0	0	0	0	0
Medullary carcinoma (Typical)	01	0	0	0	0	1	100	0	0

(p > 0.05)

Invasive carcinoma of no special type was the commonest type (60/66, 90.90%).

Table 4 - HER-2/neu status according to grade of tumor (n=66)

Grade	Total number of cases	HER-2/neu							
		Negative				Equivocal		Positive	
		Score 0		Score 1+		Score 2+		Score 3+	
		n	%	n	%	n	%	n	%
I	19	12	63.15	4	21.05	1	5.26	2	10.52
II	32	11	34.37	10	31.25	5	15.63	6	18.75
III	15	8	53.33	2	13.33	1	6.66	4	26.66

(p>0.05)

Majority of cases were grade II. There is increase in HER-2/neu positivity from grade I to grade III but it was not statistically significant.

TABLE 5- HER-2/neu status according to lymph node positivity (n=44)

Lymph Node	Total number of cases	HER-2/neu							
		Negative				Equivocal		Positive	
		Score 0		Score 1+		Score 2+		Score 3+	
		n	%	n	%	n	%	n	%
Lymph Node Positive	25	7	28.00	7	28.00	2	8.00	9	36.00
Lymph Node Negative	19	10	52.63	5	26.31	4	21.05	0	0

(p=0.01)

TABLE 6- HER-2/neu status according to NPI (n=44)

Prognostic Group	NPI	Total number of cases	HER-2/neu							
			Negative				Equivocal		Positive	
			Score 0		Score 1+		Score 2+		Score 3+	
			n	%	n	%	n	%	n	%
Good	< 3.4	5	4	80	1	20	0	0	0	0
Moderate	3.4-5.4	21	7	33.33	7	33.33	5	23.80	2	9.52
Poor	> 5.4	18	6	33.33	5	27.77	1	5.55	6	33.33

(p > 0.05)

Majority of cases were in moderate prognostic group followed by poor prognostic group.

TABLE 7- Comparison of HER-2/neu status with estrogen receptor (ER) and progesterone receptor (PR)

HER-2/neu Score	Number of cases	ER				PR			
		Positive		Negative		Positive		Negative	
		n	%	n	%	n	%	n	%
0	31	16	51.61	15	48.38	12	38.70	19	61.29
1+	16	9	56.25	7	43.75	9	56.25	7	43.75
2+	07	6	85.71	1	14.28	4	57.14	3	42.85
3+	12	7	58.33	5	41.66	5	41.66	7	58.33

HER-2/neu was positive in 12/66 (18.18%) cases. ER was positive in 38/66 (57.57%) cases. PR was positive in 30/66 (45.45%) cases. Triple negative cases were 16/66 (24.24%). Triple positive cases were 4/66 (6.06%).

Discussion

Breast cancer is one of the most common malignancies in women. Hormone receptors (ER, PR) and HER-2/neu status have a great influence on the clinical role in the proliferation and progression of breast cancer.⁽¹²⁾ The role of hormone receptors as prognostic and therapeutic tools has widespread acceptance in the management of breast cancer. The amplification of HER-2/neu gene is associated with poor prognosis, shorter time of relapse, metastasis, and therapy with trastuzumab (Herceptin).⁽¹³⁾ In the present study, HER-2/neu over – expression was compared with various clinical and prognostic parameters.

Association of HER-2/neu with Age

In the present study 59.09% patients were more than 45 years of age. HER-2/neu expression correlated with age above 45 years ($p=0.035$). In Ray et al⁽¹⁴⁾, Yadav et al⁽¹⁵⁾ study and Chand, Garg, Singla, Rani⁽¹⁶⁾ also found significant correlation between age of patient and HER-2/neu expression ($p=0.000$), HER-2/neu over expression was significantly higher among post –menopausal in comparison to pre-menopausal women. Whereas no significant association between HER-2/neu expression and menopausal status was found by Rashed, Ragab, Galal⁽¹⁷⁾.

HER-2/neu and size of tumor

In the present study, no association with size of tumor and HER-2 was found. Several studies have found no association between HER-2 over-expression and tumor size^(15,18,19,20). Whereas Ayadi et al⁽²¹⁾ showed a tendency of HER-2/neu over-expression to be more associated with larger tumor size although this difference was not statistically significant. They observed that tumor larger than 5 cm tended to have higher rates of HER-2/neu over expression than those below 5 cm in size (27.00% vs 15.3%).

HER-2/neu and Histologic Type

In the present study invasive carcinoma of no special type was the commonest histomorphological type. HER-2 positivity (Score 3+) was seen only in invasive carcinoma of no special type. Invasive lobular carcinoma, carcinoma with neuroendocrine differentiation and mucinous carcinoma were negative for HER-2/neu. There was one case of medullary carcinoma (Typical) that showed equivocal (Score – 2+) positivity. There was no statistically significant relation between HER-2 and histological type in this study. Ayadi et al⁽²¹⁾, also found no statistically significant correlation between HER-2 over – expression and histologic type. Nikhra et al⁽²²⁾ also found invasive lobular carcinoma to be negative for HER-2/neu. Lobular carcinoma has good prognosis and better response to hormonal therapy. Lee et al⁽²³⁾, and Puvitha and Shifa⁽²⁴⁾, observed HER-2/neu negativity in neuroendocrine tumor of the breast. Diab et al⁽²⁵⁾, and Puvitha and Shifa 2016 had observed HER-2 negativity in mucinous carcinoma breast. All these studies correlated with the present study. In the present study medullary carcinoma showed equivocal expression, where as most of the workers in the past have reported it to be HER-2 negative⁽²⁴⁾. The case of medullary carcinoma in this study showed equivocal result for HER-2/neu. So it needs to be further confirmed by fluorescent in situ hybridisation for its positivity or negativity.

HER-2/neu and Histological Grade

There was apparent increase in HER-2/neu over expression but statistically it was insignificant ($p > 0.05$). Reddy and Mithraa,⁽²⁰⁾ Yadav⁽¹⁵⁾ et al and Azam⁽²⁶⁾ et al, also observed that HER-2/neu expression did not reveal a significant association with tumor grade.

HER-2/neu and TNM Stage

In this study lymph nodes with metastatic deposits were more 25/44 in comparison to lymph node negative cases (19/44). When HER-2/neu expression was compared with lymph node positivity, it was observed that positive score for HER-2/neu was only seen in lymph node positive cases, whereas equivocal score was seen more in lymph node negative cases. It was found to be statistically significant in this study ($p \leq 0.05$). The findings are in concordance to a large number of workers in the past.^(24,27,28,29)

HER-2/neu and Nottingham Prognostic Index (NPI)

In the present study 42.85% cases were in the bad prognostic group which is slightly lesser than reported by previous workers. Kurshumliu et al⁽³⁰⁾, also reported that over-expression of HER-2/neu is more frequent in moderate and poor NPI groups.

Comparison of HER-2/neu with hormonal status in breast carcinoma cases

In the present study in HER-2/neu negative cases, ER was positive in 53.19% cases and PR was positive in 44.68% cases. An inverse association had been found between HER-2/neu over-expression and the presence of receptors for

steroid hormones estrogen and progesteron in both clinical correlative studies and experimental models; thus the higher the level of HER-2/neu over-expression the lower the corresponding level⁽³¹⁾ Rashed, Ragab and Galal⁽¹⁷⁾ also demonstrated an inverse correlation between HER-2 over-expression and ER and PR status. Yadav et al,⁽¹⁵⁾ Chand et al,⁽¹⁶⁾ Reddy and Mithraa⁽²⁰⁾ and Puvitha and Shifa,⁽²⁴⁾ also reported an inverse relation between ER, PR receptor and over expression of HER-2/neu. In the present study 16 cases out of 66 cases were triple negative (24.24%). Studies from Western Countries have shown that triple negative tumors constituted 14.0 – 29.5% of breast carcinoma.^(32,33,34) Studies have also shown that triple negative tumors vary markedly with ethnicity and have documented a higher incidence in African women compared to white women. In this study, 4 cases were triple positive Puvitha and Shifa,⁽²⁴⁾ Francis et al⁽³⁵⁾, Bhargava et al⁽³⁶⁾ and also reported hybrid ER/PR and HER-2/neu expressions.

Conclusion

Strong HER-2/neu (Score 3+) positivity was seen in 12/66 (18.18%) cases, moderate or equivocal (Score 2+) positivity was seen in 7/66 (10.60%) cases, mild HER-2/neu positivity (Score 1+) was seen in 16/66 (24.24%) cases and Score 0 was seen in 31/66 (46.97%) cases. A statistically significant association of HER-2/neu positivity (Score 3+) was found in patients more than 45 years of age ($p = 0.035$) and in lymph node positive cases ($p=0.01$). No statistically significant association of HER-2/neu positivity (Score 3+) was found with size, morphological type, grade of tumor & NPI Index ($p > 0.05$).

Ethics approval and consent to participate

Institutional ethics clearance was taken before starting the study and written and informed consent was taken from the patients participating in the study.

Competing interest

There is no competing interest.

Funding statement

NIL

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