RESEARCH PAPER | Medical Science | Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

Immunohistochemical Diagnosis of Breast Cancer Cases with Prognostic Markers: ER, PR & HER2/1 Neu.

KEYWORDS: Breast cancer, Estrogen /progesterone receptor, HER2/neu, Immunohistochemistry.

Arup Bhaumik
Assistant Professor and P. I. (DBP-Project) Department of Pathology Agartala Government Medical College P. O. Kunjaban, Agartala PIN: 7990006

Shikha Das
Professor and HOD Department of Pathology Agartala Government Medical College Agartala

Sandeep Roy Sarkar
Senior Research Fellow Department of Biotechnology-Project Department of Pathology Agartala Government Medical College Agartala

Payel Chakraborty
Research Assistant Department of Biotechnology-Project Department of Pathology Agartala Government Medical College Agartala

Biswajit Chowdhury
Laboratory Assistant Department of Biotechnology-Project Department of Pathology Agartala Government Medical College Agartala

ABSTRACT *Immunohistochemistry is increasingly used in the assessment of markers for breast cancer prognosis. A hospital based study was done to scrutinize the various histopathological facets of breast cancer patients along with endocrine therapy. The size of the sample was 55, ranging in age group between 22 to 70 years female patients of Tripura. The most common scoring method used was the H-score which takes into consideration the staining intensity in conjunction with the percentage of cells staining positively in breast carcinoma tissue.They were further divided into estrogen receptor sensitivity, progesterone receptor sensitivity and HER2/neu sensitivity. Results are presented to formulate ultimately a strategy for sustainable integrative model in the management of breast cancer. Our study supports IHC classification as a clinical tool,since ER, PR & HER2/neu testing is widely available at a reasonable cost.*

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer accounting for 23% of total cancer cases and 14% of cancer deaths. The incidence in India is also alarming. Every year about 1.5 lakh new cases of breast cancer have been reported by World Health Organization in India.

Immunohistochemistry (IHC) is now the globally accepted methodology for detection of Estrogen (ER) and Progesterone (PR) receptors in breast carcinomas [1]. Both ER and PR show nuclear expression in positive cases. ER content, in particular, is correlated with prolonged disease-free survival and increased likelihood of response to hormonal therapy. PR expression is reported along with ER expression, and IHC determination of PR expression has now been clinically validated [2].

Steroid hormone receptor is an important prognostic and predictive marker for response to endocrine therapy in the management of breast cancer. Several studies have found that up to 10% of estrogen receptor negative (ER–) breast cancers are progesterone receptor positive (PR+) [3]. Although recent evidence shows that the percentage is much lower when more sensitive immunohistochemical (IHC) methods for ER determination are used [4-6]. The ER and PR should be reported in combination with the HER-2 status, since these clinical phenotypes possess discriminative prognostic information, and are able to challenge gene expression profiling [6-8].

The aim of this study is to determine the status of ER, PR and HER2/neu of breast carcinoma cases.

METHODOLOGY

The retrospective study included 55 breast cancer patients diagnosed in the Pathology department, Agartala Government Medical College & G.B Pant hospital, ranging from 22 to 70 yrs (mean = *44.87*) of age. The patients had undergone unilateral breast cancer treatment during 2010 - 2014. The study had accorded ethical clearance from Institutional Ethical Committee, Agartala Government Medical College, Agartala.

The clinical history & pathology report from all the patients were reviewed. Histologic assessment of tumor type and grade were performed routinely on 4 to 5 µm thick hematoxyline & eosin stained sections of formalin-fixed, paraffin-embedded tumors according to the criteria outlined in the World Health Organization Classification of Tumors. After staining the cytopathological data was obtained from microscopic observations.

Tissue sections (4-5 µm thick) were used for all immunohistochemical analyses. Two rabbit monoclonal antibody (ER and PR) and one mouse monoclonal antibody (HER2/neu) were used for immunohistochemical analyses of breast carcinoma cases. according to the manufacturer's recommendation. The ER & PR results were screened manually and interpreted as positive when more than 10% of tumor cells showed positive nuclear staining. The HER2/neu results were interpreted as positive when tumor cells showed positive cell membrane staining.

The quick score method [9] involves the addition of scores for staining intensity and the proportion of positive cells which is a departure from the H-score which is based on multiplication of these components and is more appropriate if staining intensity reflects antigen concentration. For H-score assessment [10], ten fields were chosen at random at 40X magnification and the staining intensity in the ma-

Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

lignant cell nuclei was scored as 0, 1, 2, or 3 corresponding to the presence of no stain, weak, moderate, and strong brown staining, respectively. Each field is 218 X 163 µm. The total number of cells in each field and the number of cells stained at each intensity were counted.

Statistical analysis by Chi^2 test was performed at degree of freedom =1 and at 5% level to test the independence of two attribute, age and hormone receptor status, using MS Excel.

RESULTS

A total of 55 breast carcinoma cases were identified from GB Pant Hospital. Among them 18.18% are ductal carcinoma, 5.45% are medullary carcinoma, 9.09% cases are lobular carcinoma, 12.73% are adenocarcinoma, 45.45% are infiltrating ductal carcinoma, 9.09% cases are invasive ductal carcinoma (Table-1 and Figure-1). The age of the subjects enrolled here ranged from 22-70 years. The group of young women with breast cancer (<40) included 19 patients (34.55%). The group of young women with breast cancer (>40) included 36 patients (65.45%).

Table1: Patients and types of carcinoma

Carcinoma type	Patients (no.)	Age in years (range)	%
Ductal	10	27-60	18.18
Medullary	3	22-50	5.45
Lobular	5	37-60	9.09
Adenocarcinoma	7	23-60	12.73
Infiltrating ductal	25	31-70	45.45
Invasive ductal	5	35-55	9.09
Total	55	22-70	99.97

Table 2 demonstrates that among the 55 subjects 18 (32.73%) are ER+/PR+/ HER2 neu— subtype, 2 (3.64%) are ER— / PR+/ HER2 neu— subtype, 5 (9.09%) are ER— / PR—/ HER2 neu+ subtype, 4 (7.27%) are ER+ / PR—/ HER2 neu— subtype and 26 (47.27%) ER— / PR—/ HER2 neu— subtype. Percentage distribution of different receptor subtypes is also represented in Figure 2. Representative immunostaining for ER, PR and HER2 are shown in Figure 3.

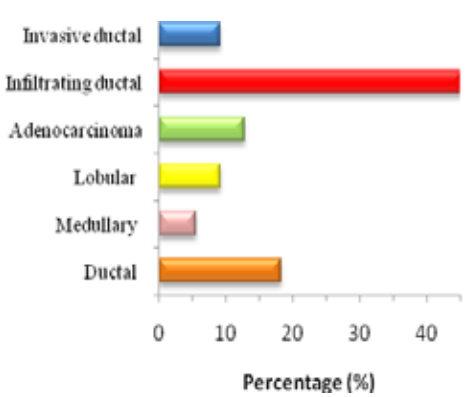

Figure 1: Percentage distribution of different types of carcinoma

Table 3 shows distribution of ER/PR and Her2 receptor status. Estrogen receptor status was statistically not significant ($\chi^2 = 0.05$) between less than 40 yrs and more than 40 yrs of age. Progesterone receptor status was not statistically significant ($\chi^2 = 0.29$) between less than 40 yrs and more than 40 yrs of age. Similarly, Human epidermal growth factor receptor 2 (HER2/neu) receptor status was not significant ($\chi^2 = 0.07$) between the two age classes. We conclude that age and receptor status are independent, i.e., they are not associated.

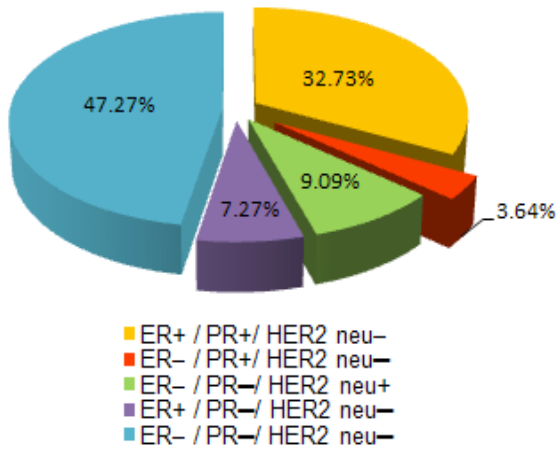

Fig 2: Receptor status of Breast Cancer

DISCUSSION

Immunohistochemistry is now a globally accepted methodology for detection of Estrogen, Progesterone, and HER2neu receptors in breast carcinoma cases for response to endocrine therapy in the management of breast cancer. This study confirmed breast cancer as a multifaceted disease comprising of distinct biological subtypes with diverse natural history which are increasingly recognized as presenting a varied spectrum of clinical, pathologic and molecular features with different prognostic and therapeutic implications [11]. Our results reveal statistically significant differences in clinical and pathologic features and outcomes between subtypes. Recent studies have demonstrated that ER expression is present in approximately 70% of breast cancers, so an accurate and reliable ER result is critical for hormone therapy [12]. PR expression is generally reported along with ER expression. It has further been suggested that PR status is independently associated with disease-free and overall survival, that is, patients with ER-positive/PR-positive tumors have a better prognosis than patients with ER-positive/PR-negative tumors, who in turn have a better prognosis than patients with ER-negative/PR-negative tumors. PR analysis can provide important prognostic information and prediction of response to adjuvant hormone therapy in ER positive tumors [5].

Table 2: ER/PR and Her2 status of Breast Cancer cases.

Carcinoma type	ER+ / PR+/ HER2 neu–	ER– / PR+/ HER2 neu—	ER– / PR—/ HER2 neu+	ER+ / PR—/ HER2 neu—	ER– / PR—/ HER2 neu—
Ductal	4	1	2	-	3
Medullary	-	-	1	-	2
Lobular	-	1	1	1	2
Adenocarcinoma	4	-	-	-	3
Infiltrating ductal	9	-	-	3	13
Invasive ductal	1	-	1	-	3
Total	18	2	5	4	26

Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

Table 3: Age distribution and receptor status

	ER			PR			HER2/neu		
	Age < 40 yrs	Age > 40 yrs	Total	Age < 40 yrs	Age > 40 yrs	Total	Age < 40 yrs	Age > 40 yrs	Total
Positive	8 (14.54)	14 (25.45)	22	6 (10.90)	14 (25.45)	20	2 (3.63)	3 (5.45)	5
Negative	13 (23.63)	20 (36.36)	33	13 (23.63)	22 (40)	35	17 (30.90)	33 (60)	50
Total	21	34	55	19	36	55	19	36	55

Degrees of freedom=1 Figures in the parentheses are percentage.

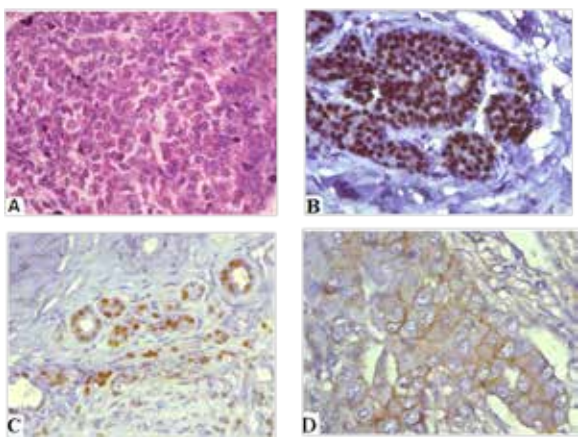

Figure 3: Photographic illustration of ER, PR and HER2 neu positive cases
A. Hematoxyline & Eosin stain of Breast carcinoma.
B. Strong nuclear staining of ER.
C. Weak nuclear stain of PR.
D. Moderate membrane staining of HER2 neu.

As with all IHC studies of therapeutic targets, accurate and perhaps quantitative assessment of the results is critical. There are several major factors that can dramatically affect the apparent ER and PR status of a breast carcinoma as determined by IHC, and determination of thresholds for reporting immunostaining and reproducibility [13].

Technical issues in performing IHC can potentially change steroid receptor results, adversely affecting patient care. Awareness of this issue will guide researchers to re-evaluate their validation studies that are currently in use, and will prompt pathologists to repeat ER/PR tests if the results do not correlate with histology, particularly in cases of low grade carcinomas. Our results reveal statistically significant differences in clinical and pathologic features and outcomes between subtypes.

In case of H- score method, an H-score between 0 and 300 was obtained where 300 was equal to 100% of the tumor cells showed strong reactivity. H-score gives us maximum score of 300 if 100% cells show strong reactivity. Use of H score method which is a semi quantitative method, produce a numerical score influenced by intensity of the reactivity, with the amount of receptor present. It helps us to treat cancer. Using such a simple scoring method we can know that,

Table 4: H scoring of ER and PR.

H score	ER	PR
0-100	6 (27.27)	12 (60)
101-200	8 (36.36)	8 (40)
201-300	8 (36.36)	0 (0)
Total no of cases	22 (100)	20 (100)

Figures in the parentheses are percentage.

score of 0 indicates that endocrine therapies will not work. So the patient should receive an alternate therapy as first line therapy. If score is 300, indicates that endocrine therapies will work, so the patient can be treated with endocrine therapy. Table-4 represents the H score status of our studied samples. Mean of the positive % of cells are 88.41 (ER) and 57.85 (PR).

CONCLUSION

The expression of the Immunohistochemical study (ER, PR & HER2/neu markers) was an independent prognostic factor for outcome in Breast cancer patients. The results from this study would be used to help developing diagnostic and treatment strategies that are based on the risk factors of the individual patient. IHC classification is a clinical tool using ER, PR & HER2/neu testing which is widely known as endocrine therapy, available at a reasonable cost compared to chemotherapy & radiotherapy

ACKNOWLEDGEMENT

This work has been supported and funded by the Department of Biotechnology (DBT) Project, Govt. of India, New Delhi.

REFERENCE

1.Harvey, J. M., Clark, C. M., Osborne, C. K., Allred, D. C. (1999). Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. Journal of Clinical Oncology, 17, 1474–1481. | | 2. Mohsin, S. K., Weiss, H., Havighurst, T., Clark, G. M., Berardo, M., Roanh, le.D., et al.(2004). Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. Modern Pathology, 17, 1545–1554. | | 3. Rakha, E. A., Reis-Filho, J. S., Ellis, I. O. (2010). Combinatorial biomarker expression in breast cancer. Breast Cancer Research & Treatment,120(2), 293–298. | | 4. Rhodes, Jasani, A. B. (2009). The oestrogen receptor-negative/progesterone receptor-positive breasttumour: a biological entity or a technical artifact. Journal of Clinical Pathology, 62, 95-96. | | 5. Rakha, E. A., El-Sayed, M. E., Green,A. R., etal.(2007). Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. Journal of Clinical Oncology,25(30), 4772-4778. | | 6. Rakha, E. A., Reis-Filho, J. S., Ellis, I. O. (2010). Combinatorial biomarkerexpression in breast cancer. Breast Cancer Research & Treatment, 120(2), 293-308. | | 7. Brouckaert, O., Pintens, S., Van, Belle, V.,etal.(2009). Short-term outcome of primary operated early breast cancer by hormone and HER-2 receptors. Breast Cancer Research & Treatment, 115(2), 349–358. | | 8. Hammond, M. E., Hayes, D. F., Wolff, A. C.,etal.(2010). American society of clinical oncology/ college of American pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Journal of OncologicalPractice, 6(4), 195–197. | | 9. Barnes, D. M., Dublin, E. A., Fisher, C. J., Levison, D. A., Millis, R. R. (1993). | Immunohistochemical detection of p53 protein in mammary carcinoma. Human Pathology, 24, 469-476. | | 10. Detre, S., Jotti, G. S., Dowsett, M. (1995). A quickscore method forimmunohistochemical semiquantitation:validation for oestrogen receptor in breastcarcinomas. Clinical Pathology, 48, 876-878. | | 11.Carey, L. A., Perou, C. M., Livasy, C. A., Dressler, L. G., Cowan, D., Conway, K., et al.(2006).Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. TheJournal of the American Medical Association, 295, 2492-2502. | | 12. Nadji, M., Gomez-Fernandez, C., Ganjei-Azar, P., Morales, A. R. (2005). Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. American Journal of Clinical Pathology, 123, 21–27. | | 13. Gown, A. M. (2008). Current issues in ER and HER2 testing by IHC in breast cancer. Modern Pathology,21(2), S8–S15. |