ORIGINAL RESEARCH

Breast cancer histomorphology and it's correlation with ER/PR, HER-2 NEU and P-53 expression

[1]Dr. Neelima Verma, [2]Dr. Saumya Singh Rajput

[1]Professor, [2]Junior Resident, Department of Pathology, GSVM Medical College, Kanpur, Uttar Pradesh, India

Corresponding Author
Dr. Saumya Singh Rajput
Junior Resident, Department of Pathology, GSVM Medical College, Kanpur, Uttar Pradesh, India

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ABSTRACT

Background: Breast carcinoma is the most common malignant tumour and the leading cause of carcinoma death in women, with more than 2.1 million cases occurring worldwide annually. Currently, routine clinical management of breast cancer incorporates specific molecular markers; namely ER- estrogen receptor, PR-progesterone receptor, HER2-human epidermal growth factor receptor 2 gene, that have been proven to provide therapeutic, predictive and prognostic value. Hence, the present study is being undertaken to analyze the status of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu) and p53; in all these breast carcinomas in respect to histomorphology and lymph node status. **Methods:** The study included retrospective and prospective cases from Jan 2021 to Oct 2022. After obtaining the informed consent, the study included all the lumpectomy, simple mastectomy and modified radical mastectomy specimens of clinically diagnosed breast malignancy in female of all age group, submitted to the Department of Pathology, GSVM Medical College Kanpur for histopathological study. The histological and IHC was done as per hospital SOPs. The results were analysed using descriptive statistics and making comparisons among the various groups. Categorical data were summarized as in proportions and percentages (%) while discrete in mean and SD. **Results:** A total of 100 cases of infiltrating breast carcinoma were included in this study. 56% cases belonged to age group of 41-50 years. ER positivity was observed in 68% [68/100] (Figure 1). PR positivity was observed in 38% of cases [38/100]. Her 2neu positivity was observed in 34% of cases (34/100) and Her 2 neu negative was observed in 66% of cases (66/100). Positive P53 staining i.e. more than 10% cells was observed in 28 of cases (28%) and negative in 72 cases (72%). ER and PR positivity were higher in postmenopausal patients 61.8% and 68.4% respectively. Her 2neu positivity was high in postmenopausal group i.e. 82.4%. P53 expression was also high in postmenopausal patients i.e. 89.2%. **Conclusion:** The expression of the Immunohistochemical study (p53, ER, PR & HER2/neu markers) is 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.

Keywords: Breast, Cancer, histomorphology, lymph nodes, postmenopausal.

INTRODUCTION

Breast carcinoma is the most common malignant tumour and the leading cause of carcinoma death in women, with more than 2.1 million cases occurring worldwide annually. In our country, though the incidence of breast carcinomas is lower than the west yet it is the second most common malignant tumour in females comprising 14 to 16%, the first being carcinoma cervix. Indian females with age adjusted rate as high as 25.8 per 1,00,000 women and mortality 12.7 per 1,00,000 women, data reports from various latest national cancer registries were compared for incidence and mortality rates (1,2).

Currently, routine clinical management of breast cancer incorporates specific molecular markers; namely ER- estrogen receptor, PR-progesterone receptor, HER2-human epidermal growth factor receptor 2 gene, that have been proven to provide therapeutic, predictive and prognostic value. Studies have shown that in contrast to western literature, percentage of estrogen and progesterone receptor (ER and PR) positive tumors in Indian subcontinent is persistently low (3,4). There is a need to evaluate the expression of HER-2, estrogen receptor (ER) and progesterone receptors (PR) in breast carcinoma and to analyze it with the histomorphology. For accessing ER; PR status in immuno-histochemistry, Allred

scoring is very helpful. The IHC test gives a score of 0 to 3+ that measures the amount of HER2 receptor protein on the surface of cells in a breast cancer tissue sample (5,6).

p53 functions in immunity by induction of apoptosis, removal of apoptotic cells, antiviral defence, induction of type I IFN, enhanced pathogen recognition, cytokine production, and immune checkpoint regulation. Several studies have explored the association of p53 and tumour immune regulation (7,8,9). Hence, the present study is being undertaken to diagnose cases of carcinoma breast on histopathological examination and to access lymph-node status/ involved by breast carcinoma.Also, to analyze the status of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu) and p53; in all these breast carcinomas in respect to histomorphology and lymph node status.

MATERIALS and METHODS

The study included retrospective and prospective cases from Jan 2021 to Oct 2022. After obtaining the informed consent, the study included all the lumpectomy, simple mastectomy and modified radical mastectomy specimens of clinically diagnosed breast malignancy in female of all age group, submitted to the Department of Pathology, GSVM Medical College Kanpur for histopathological study. The study excluded patients with metastatic malignancy of breast and already treated for contralateral breast cancer.

PROCEDURE

The Specimen received in 10% formalin was put in 10% formalin after loafing for 6-8 hr. Grossing was performed by taking the samples from nipple areola complex, all 4 margin (label it mentioned), base, growth and lymph nodes (if received) and the cassettes was sent for tissue processing and block formation. When blocks were formed, a paraffin sections of 5µm thickness were stained by hematoxylin and eosin (H & E) for histopathological study.

For immunohistochemistry, sections of 3µm taken on charged/coated slides with poly -L-lysine. The slides were put on the hot plate for dewaxing for 1 hr and as dewaxing was completed, dip the slides was dipped into xylene directly from hot plate. The slides were kept in the xylene for 10 min and were transferred into second xylene solution for 10 min.After that the slides was passed into downgrading concentration of alcohol in order of 100%, 70%, and 50% for 5 min in each and the slides were washed with distilled water for 5-10 min.Next step was antigen retrieved (ER, PR, HER-2, NEU, and p53) by the citrate buffer (pH-6) at high temperature and high pressure and when retrieval was completed, the slides were put in moist chamber and allowed to it cool down to room temperature for app. 30 min. The slides were washed with TRISS buffer (pH-7.5) (2-3 times) and the peroxidase blocker was added to each section for 15 min.

The washing was done 3 times with TRISS buffer andthe slides were labelled as ER, PR, HER-2, NEU, and p53,and the slides were incubated with respective primary antibody for 1 hr. The washing was done 3 times with TRISS buffer and the slides were incubated with secondary antibody HRP (horse radish peroxidase) for 30 min. The washing was done 3 times with TRISS buffer and DAB chromogen was mixed in DAB buffer in the ratio of 1: 50 and applied to it for 30 mins. Now 3 washing of distilled water was given and the slides were counterstained with hematoxylin for appropriate time (3-5min). Lastly, 3 washing of distilled water was given and the slides were dried and mounted with DPX and microscopy was done.

The data was collected from the cases in a pretested proforma, which included patient details such as age, sex, occupation, chief complaints, personal history (smoking, tobacco, alcohol intake), family history, investigation history (X ray, CT scan, MRI, Endoscopy, Biopsy), treatment History (types of therapy, surgery), gross examination (site, appearance, size,invasion, nodal metastasis), histological features and IHC findings [ER Positive, ER Negative, PR Positive, PR Negative, p53 positive, p53 negative, HER2/Neu (+ve) and HER2/Neu (-ve)].

STATISTICAL ANALYSIS

The results were analysed using descriptive statistics and making comparisons among the various groups. Categorical data were summarized as in proportions and percentages (%) while discrete in mean and SD.

RESULTS

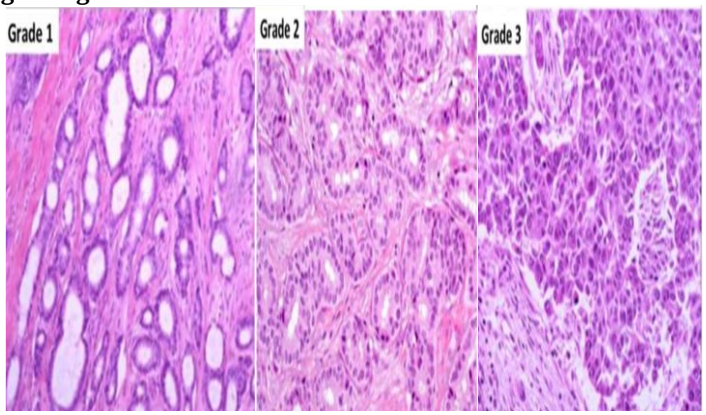
A total of 100 cases of infiltrating breast carcinoma were included in this study. 56% cases belonged to age group of 41-50 years. Mean age was 48.76 years. Out of 100 cases, 46 cases (46%) were premenopausal and 54 cases (54%) post-menopausal. 76 cases (76%) had tumor size between 2-5 cm, 16 cases (16%) had tumor size more than 5 cm and 8 cases (8%) had tumor size between < 2 cm. In 54 cases, metastatic lymph node was present, out of 54 cases, 20 cases having 4 or more metastatic lymph node positive (Table 1).

Table 1: Distribution of cases on the basis of baseline characteristics

Variables	Frequency	Percentage
Age group (in years)		
0-30	2	2%
31-40	12	12%
41-50	56	56%

51-60	18	18%
61-70	10	10%
> 70	2	2%
Menopausal status		
Pre-menopausal	46	46%
Post-menopausal	54	54%
Tumor size (in cm)		
< 2	8	8%
2– 5	76	76%
> 5	16	16%
Lymph node status		
Not identified	14	14%
Negative	32	32%
Positive (1-3 nodes)	34	34%
Positive (4 or more)	20	20%

Figure 1.Histological grading of the cases

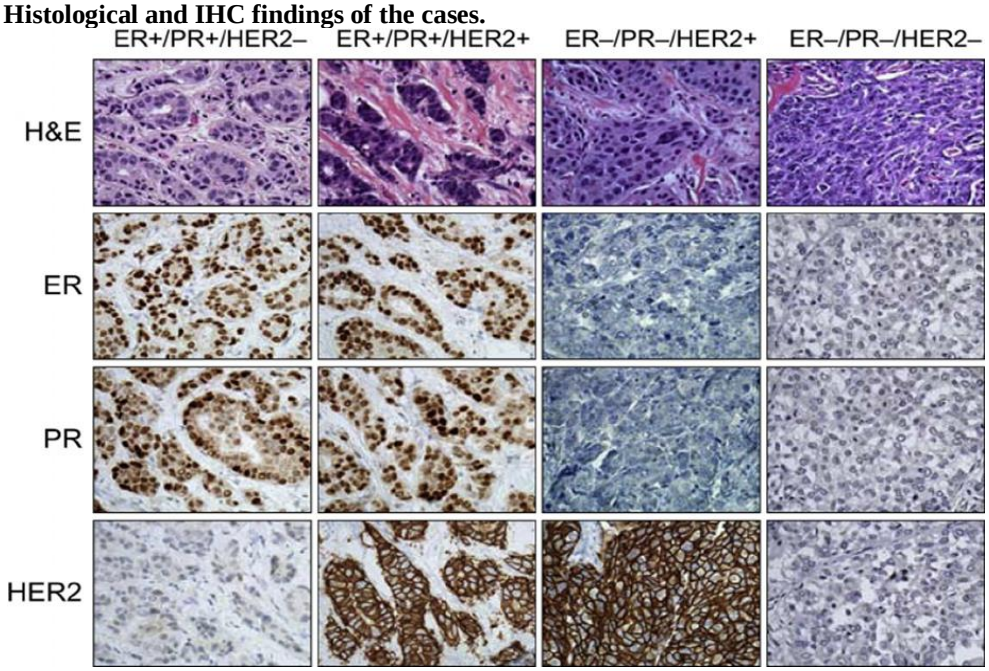


There were 92 cases (92%) were diagnosed invasive ductal carcinoma, 4 cases of invasive lobular carcinoma, 2 cases of medullary carcinoma. 2 cases of mucinous carcinoma. In Bloom Richardson grading, 62% cases belong to grade II, 30% cases belong to grade III and 8% cases belong to grade I. ER positivity was observed in 68% [68/100] (Figure 1). PR positivity was observed in 38% of cases [38/100]. Her 2neu positivity was observed in 34% of cases (34/100) and Her 2 neu negative was observed in 66% of cases (66/100). Positive P53 staining i.e. more than 10% cells was observed in 28 of cases (28%) and negative in 72 cases (72%) (Table 2).

Table 2: Distribution of the cases on the basis of histological and IHC findings

Variables	**Frequency**	**Percentage**
Histological types		
Invasive ductal carcinoma	92	92%
Invasive lobular carcinoma	04	4%
Medullary carcinoma	02	2%
Mucinous carcinoma	02	2%
Histological Grades		
Grade I	08	8%
Grade II	62	62%
Grade III	30	30%
IHC Status	**Positive**	**Negative**
ER	68	32
PR	38	62
HER 2 NEU	34	66
P53 immunostaining	28	72

Figure 2: Histological and IHC findings of the cases.



ER and PR positivity were higher in postmenopausal patients 61.8% and 68.4% respectively. Her 2neu positivity was high in postmenopausal group i.e. 82.4%. P53 expression was also high in postmenopausal patients i.e. 89.2%.For T1 stage, out of 8 cases, 6 were ER +ve, 2 was ER -ve , PR +ve 6, and PR -ve 2 cases. P53 positivity seen in 1 case of T1, 19 cases of T2, and 8 cases of T3. No significant pattern seen in Her2neu expression and tumor size. As IDC NOS constituted the bulk of cases (92 cases) out of 100, 4cases of lobular carcinoma, 2 case of medullary carcinoma and 2 case of mucinous carcinoma. ER and PR negativity increased with grade of the tumour (Figure 2). Her2neu positivity decreased with tumor grade. P53 positivity is significantly correlated with a higher grade of the tumour. There was not much of correlation between the nodal status and ER/PR/ Her2neu and P53 receptor status (Table 3).

Table 3: Distribution of the cases on the basis of IHC findings for various patients characteristics.

Variables	ER $^{+}$	ER^{-}	PR $^{+}$	PR $^{-}$	Her2neu^{+}	Her2neu^{-}	P53^{+}	P53^{-}
Menopausal status								
Pre-menopausal	26	20	12	34	06	40	03	43
Post-menopausal	42	12	26	28	28	26	25	29
Tumor size (in cm)								
< 2	6	2	6	2	7	1	1	7
2– 5	58	18	28	48	15	61	19	57
> 5	4	12	4	12	12	4	8	8
Histological Type								
IDC NOS	62	30	32	60	32	60	24	68
ILC	04	00	04	00	02	02	02	02
Medullary	00	02	00	02	00	02	00	02
Mucinous	02	00	02	00	00	02	02	00
Tumour grade								
I	08	00	06	02	06	02	01	07
II	52	10	24	38	20	42	15	47
III	08	22	08	22	08	22	12	18
Lymph node								
Negative	24	08	04	28	04	28	06	26
Positive	26	28	20	34	14	40	10	44

ILC: Invasive lobular carcinoma

DISCUSSION

Immuno histochemistry 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.

Our study comprised 100 cases of invasive breast carcinoma with maximum incidence of breast cancer (56%) was observed in the age-group of 41-50 years. This is in accordance with work done by Kaur et al., who noted a peak age of 45 - 55 years in a study done on 177 cases (10).

In our study, in total of 100 cases, 46 % were premenopausal and rest 54% were post- menopausal. Following studies correlate with our study.which is in accordance with work done by Saleh et al., on 166 patients of breast carcinoma (11).

One of the most important and well-established prognostic factors in carcinoma breast is tumor size (12,13). In our study 76 (76%) cases have tumor size 2-5 cms, 16 (16%) cases have tumor size > 5 cms, 08(8%) cases have tumor size less than 2 cms. This is in concurrence with results obtained by Kaur et al., and Saleh et a., (10,11), where as in the western countries, Taucher et al., reported that the tumors were predominantly less than 2 cm in size which could be due to early detection programs prevalent in the western countries (14).

Tumor grade is another well-established prognostic marker in case of breast carcinoma (15).In our study, 62% cases belong to grade II of Bloom Richardson grading, while 30% and 8% cases belonged to grade III and grade I respectively, which is in accordance to findings noted by Hanif et al., who reported maximum number of tumors (59%) to be of grade II (16). Maximum number of grade II cases were also noted in other studies (17,18). This difference in the tumor grades may be due to lack of routine mammographic screening in our population coupled with the lack of awareness.

In our study out of 100 cases, 54 cases are metastatic lymph node positive. Out of 54 cases, 20 cases were having 8 or more metastatic lymph node. The findings are in concurrence with the work done by Shokouh et al., (Grade 3 tumors 73.5%) (19). Kaur et al., noted that in grade III tumors lymph node metastasis was present in 57.6% cases (10).

In our study, 68% cases are ER positive cases and 32% cases are ER negative. 38% cases are PR positive cases and 62% cases are PR negative. 34% cases are Her 2 neu positive cases and 66% cases are her 2 neu negative. Indian literature reports estrogen receptor positivity varying between 30-50% (20). Manjunath et al., demonstrated that ER negative disease occurred at a younger age, at a mean of 50.2 years (SD 10.28), whereas the mean age of ER positive disease was 55.7years (21). Desai et al., from India have documented the prevalence of 46.1% for PR-positive breast cancers.[22] Ambroise et al., in their study from South India have showed 51% PR positivity (23). Similarly, Mudduwa et al., in a study from Srilanka documented a prevalence of 48.3% PR-positive tumors (24).

In our study, 28% are P53 positive and 72% are P53 negative. Out of 28% P53 positive cases, 1% positive in grade I, 15% positive in grade II and 12% positive in grade III tumor. It shows that P53 expression increases with increasing in grade of tumor. An inverse association between hormones receptors and p53 has been demonstrated by Ahmed et al., on Yemini women with breast cancer.[9] This was further confirmed by Sirvent et al., (25). Lacroix et al., showed that breast tumors expressing a high amount of p53 (as measured by IHC) are more frequently ER negative and PR-negative. They are also associated with a high proliferation rate, high histological and nuclear grades, aneuploidy and poorer survival (26).

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.

CONCLUSION

The expression of the Immunohistochemical study (p53, ER, PR & HER2/neu markers) is 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 p53, ER, PR & HER2/neu testing which is widely known as endocrine therapy, available at a reasonable cost compared to chemotherapy & radiotherapy.

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