Evaluation of ER, PR, HER2 / neu Receptor Status in Breast Cancer - A Cross Sectional Study from Manipur

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ABSTRACT

BACKGROUND

Breast cancer is the most common cancer in women worldwide and has surpassed cervical cancer in India. As a result of regular mammography screening programs and public awareness, early-stage breast cancer with better prognosis has occurred. Immunohistochemistry (IHC) is now a common practice in tumour evaluation which are of importance in diagnosis as well as for deciding the treatment and of prediction of prognosis in breast cancer. The present study was undertaken in view of correlating the histopathology of the tumour and IHC profile with respect to oestrogen receptor (ER), progesterone receptor (PR) and HER2 / neu.

METHODS

The study is a cross-sectional prospective analysis of patients with primary carcinoma of breast who are undergoing surgery over a period of 2 years from September 2017 to August 2019. All specimens were processed and stained with haematoxylin and eosin and immunohistochemistry was done for oestrogen and progesterone receptors and HER 2neu.

RESULTS

A total of 20 cases of breast carcinoma specimens were studied, whose age ranged from 24 - 72 years of age. All the 20 cases occurred in females. Infiltrating duct carcinoma was the most histological subtype noted in this study comprising 85 % (17 / 20) of cases. In the study 50 % (10/20) of the cases showed ER / PR positivity with negative HER2 / neu while 15 % (3/15) of cases were negative for ER / PR and HER2 / neu and only one case showed positivity for all three markers. 10 cases (50 %) had lymph node metastases in this study.

CONCLUSIONS

Breast cancer is the most commonly diagnosed malignancy in Indian women and is also the leading cause of cancer death in women worldwide. Assessment of receptor (ER, PR and HER − 2 / neu) status along with histopathological grading and staging will guide the clinicians to impart a correct treatment protocols to the patients. It will also be of great help in assessing the prognosis.

KEYWORDS

Breast Cancer, Histopathology, Immunohistochemistry

BACKGROUND

Breast cancer is a heterogenous disease with variable biological and clinical characteristics because of its varied genetic make-up. Most of the breast cancer commonly originates from the inner lining of milk ducts or the lobules supplying the ducts.[1] Breast cancer is still one of the leading causes of cancer death in women and worldwide. Breast cancer accounts for 23 % of all cancers in women.[2] which is also the most common invasive malignancy in women.[3] The number of cases worldwide has significantly increased since the 1970s, a phenomenon partly attributed to the modern lifestyles and now it is the most common malignancy in women around the world.

In India the mortality to incidence ratio was found to be higher in rural registries compared to urban registries and it is also ranked number one cancer among Indian females by passing cervical carcinoma.[4] Incidence in younger age group is also on the rise. Over the last few decades there has been outstanding development in breast cancer management leading to early detection of disease and improved outcome. Recent attention has been directed singularly at molecular classification of breast cancer. Molecular and genetic testing in breast cancer is of high prognostic and predictive value. However, because of the expensiveness, it is not yet widely available. Various histopathological parameters have prognostic significance in breast carcinoma. These include tumour size, histological subtype and grade, lymph node metastases, and lymphovascular invasion, oestrogen receptor / progesterone receptor status, epidermal growth factor receptor 2 / neu overexpression, proliferation activity & DNA content. Although examination of the routine haematoxylin - eosin (H & E) stained tissue sections is the gold standard for the diagnosis of breast cancer, immunohistochemistry remain an integral part of the process.[5] In breast cancer ER, PR and HER-2 represent the most acceptable markers for predicting prognosis, response or resistance to treatment.[6] Many studies suggest that clinical and pathological response to treatment varies with the pattern of expression of ER, PR and HER–2 / neu by the tumour.[7-11]

The presence of PRs, which is induced by oestrogen is also a predictor of response and determination of progesterone receptor expression has now been clinically validated.[8] Patients with ER – positive / PR - positive tumours have better prognosis then ER $_{+/}$ PR - tumours, which again have better prognosis than with ER - / PR - tumours.[9] Her–2 / neu over expression in breast cancer is usually associated with tumour aggression and poor prognosis.[12,13] However, with the availability of drugs like trastuzumab which target HER – 2 neu receptor, the outcome is markedly improved.

Triple negative breast cancer (ER -, PR -, HER – 2 / neu) are more difficult to treat and often require combination therapies as these tumours do not respond to hormone therapy. Such tumours are more likely to spread and recur. Young women have a tendency to have larger tumour size, more positive lymph node, more negative hormone receptors, and higher tumour grade than older women.[14,15]

Objectives

To determine the immunohistochemical profile in breast carcinoma using ER, PR, HER–2 / neu which could be used as prognostic factor for early management of this malignancy and to develop platform for further disease investigation and clinical management of the disease in this part of the country.

METHODS

This study is a cross-sectional prospective study of patients with primary carcinoma of breast who were undergoing surgery over a period of 2 years from September 2017 to August 2019 at Department of Pathology, JNIMS, Imphal. The study was conducted after getting clearance from institutional ethical committee (No. Ac / 06 / IEC / JNIMS / 2017 (PGT) - R. Consent were taken from each patient.

Inclusion Criteria

Patients greater than 18 years with primary breast carcinoma undergoing surgery in JNIMS Hospital from September 2017 to August 2019.

Exclusion Criteria

The patients with a second primary breast cancer and the patients who refused to participate in the research work.

After proper fixation of the specimen received in 10 % buffer formalin, multiple tissue sections were taken for each centimetre of the tumour mass after a detailed gross examination. In case of any abnormal appearing area on gross examination, an additional section was taken and processed. Paraffin embedded sections were used for both histopathological examinations and IHC.

Histopathological examination was done on haematoxylin and eosin stained slides. The stained slides were examined for histological types, vascular and lymph node involvement by the tumour, areas of surgical necrosis, desmoplasia, status of the resected surgical cut margin, lymphocytic infiltration within the tumour, presence of in-situ component, associated sclerosing adenosis, fibrocystic disease etc. Grading and staging of the tumours were done according to Nottingham's Modified Scarf Bloom Richardson score and TNM (Tumour, Node, Metastasis) staging system. For IHC, formalin fixed paraffin embedded (FFPE) tumour tissues are cut into sections of 3 - 4 micron thickness. After antigen retrieval by pressure cooker method, blocking reagents are added followed by primary and secondary antibodies. It is then stained with 3' - 3' diaminobenzidine hydrochloride (DAB) as the chromogen. Both H & E stained slides and IHC slides were reviewed and percentages and intensity of nuclear immune staining for ER & PR & membrane staining in case of HER - 2 / neu were assessed. Appropriate negative and positive controls were run with every batch of tests samples.

Immunohistochemical assessment of the status of ER and PR was done by using the Allred Score system. The proportion of tumours cells showing positive reactivity and their intensity of staining were included in the Allred Score. Adding the proportion and intensity score gives a maximum score of 8. Tumours with total scores of 3 or more were reported as "positive" and 0 to 2 score were reported as "negative".

For HER − 2 / neu (c − erbB - 2 a strong complete membrane staining observed in > 10 % of the tumour cells were reported as "Positive".

Statistical Analysis

Data collected was entered into statistical package for social sciences (SPSS) (IBM) Version 21 and descriptive statistical methods like mean, standard deviation, percentages etc. were calculated.

RESULTS

During the study, a total of 20 breast carcinoma specimens were analysed. The age of patients ranged from 24 to 72 years and the mean age at presentation was 48.9 (SD 10.05). Most of the grade I tumours were seen in patients above 60 years of age while grade 2 and 3 tumours were seen in patients below 60 years of age. Out of the 20 cases, majority of the affected side was found to be the left breast comprising 15 cases (75 %) and the remaining 5 cases (25 %) on the right breast.

No case of bilateral involvement was found. All the 20 cases occurred in females. Infiltrating duct carcinoma was the most histological subtype (Fig 1) noted in this study comprising 85 % (17/20) of cases followed by papillary carcinoma (10 %, 2/20) and a single case of medullary carcinoma (5 %).

In our study 50 % (10/20) of the cases showed ER / PR positivity (Fig 2) with negative HER2 / neu, 15 % (3/15) of cases were negative for ER / PR and HER2 / neu and only one case showed positivity for all three markers.

The study showed that grade 1 tumours exhibited 100 % positivity for ER / PR while HER − 2 / neu was negative whereas 63.6 % of the grade 2 tumours were ER / PR positive with HER − 2 / neu - negativity and 83.3 % of grade 3 tumours showed negativity for ER / PR along with a positive HER2 / neu expression (Fig 3).

Majority of these cases (6 cases, 60 %) showed HER2 / neu positivity and 2 cases (20 %) were positive for both ER & PR and triple negativity was noted in 2 cases (20 %). In this study 10 cases (50 %) had lymph node metastases.

In our study all the cases were from stage I to III and there was no case of stage IV. Majority (10 cases, 50 %) of the cases were in stage II where 60 % showed ER / PR positivity and 30 % showed HER2 / neu positivity. Out of the 7 cases in stage III, 57.14 % showed HER2 / neu positivity implying that higher stage shows more HER2 / neu positivity. We found that positive ER and PR status has an inverse association with positive HER − 2 / neu status where out of the HER − 2 / neu positive tumours (7 cases, 35 %) ER & PR

were negative in 30 % of cases while 1 case (5 %) showed triple positivity. The baseline characteristics of the breast carcinoma patients are shown in Table 1.

Variables	Characteristics	No of Cases (%)
Age	< 50	12 (60 %)
	> 50	8 (40 %)
Morphological types	Infiltrating ductal carcinoma	17 (85 %)
	Papillary carcinoma	2 (10 %)
	Medullary carcinoma	1 (5 %)
Tumour size	< 3cm	3 (15 %)
	3 - 5cm	12 (60 %)
	> 5cm	5 (25 %)
Tumour grade	I	3 (15 %)
	II	11 (55 %)
	III	6 (30 %)
Metastatic lymph node	Positive	10 (50 %)
	Negative	10 (50 %)
Lymphovascular invasion	Positive	6 (30 %)
	Negative	14 (70 %)
Tumour stage	1	3 (15 %)
	2	10 (50 %)
	3	7 (35 %)
	4	0
ER and PR status	Positive	11 (55 %)
	Negative	9 (45 %)
HER − 2 / neu status	Positive	7 (35 %)
	Negative	13 (65 %)
Triple positive	No. of triple positive cases	1 (5 %)
	No. of non-triple positive cases	19 (95 %)
Triple Negative	No. of triple negative cases	3 (15 %)
	No. of non-triple negative cases	17 (85 %)

Table 1. Baseline Characteristics of the Cases in This Study
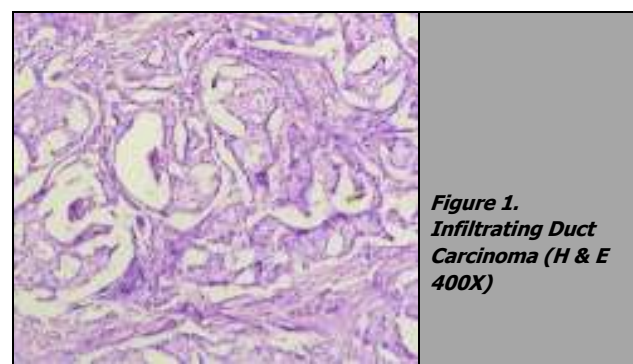

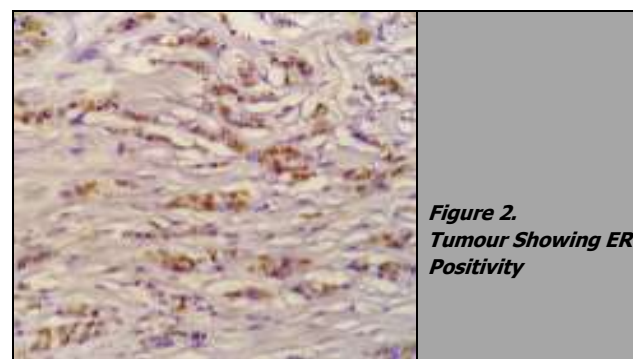*Figure 1. Infiltrating Duct Carcinoma (H & E 400X)*
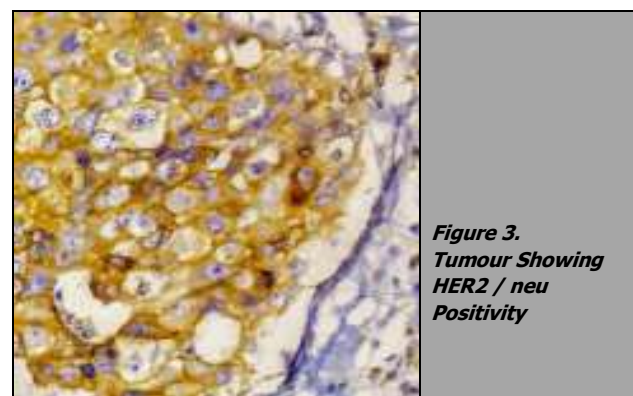

Figure 2. Tumour Showing ER Positivity

Figure 3. Tumour Showing HER2 / neu Positivity

DISCUSSION

IHC in breast carcinoma has become an integral part of complete and comprehensive histopathology report. The hormone markers – ER / PR and HER – 2 / neu have become the mainstay requirement for the oncologist where in addition to histological grade and tumour subtype, prognosis and prediction of response to treatment has become its utmost importance.

The present study was undertaken in view of correlating the histopathology of the tumour by way of histologic grade, size, lymph node status and various other prognostic factors as well as its IHC profile with respect to ER, PR, and HER – 2 / neu. This study consisted 20 cases of breast carcinoma specimens whose age ranges from 24 - 72 years of age. 12 (60 %) of the case were present below < 50 years of age and 8 (40 %) of the cases were present in above > 50 years of age.

In this study, out of 20 cases of breast carcinoma, 17 cases were of infiltrating ductal carcinoma (IDC), NOS type (85 %), followed by 2 cases of papillary carcinoma (10 %) and 1 case of medullary carcinoma (5 %) and no case of any other subtypes of breast carcinoma. These finding of IDC being the most common morphological subtypes is similar to the other studies done by UshaSharma[6] et al. (88.9 %), Bhagat Vasudha[7] et al. (99.82 %), R. Shrikant[8] et al. (83.2 %) and Adedayo[9] et al. (72.7 %). From the above studies it was concluded that the overall most common type of breast carcinoma was IDC, NOS type.

In this study majority (60 %) of the cases showed tumour size between 3 - 5 cm, followed by 25 % showing > 5 cm and 15 % showing < 3 cm. These finding of tumour size, 3 – 5 cm showing maximum number of breast carcinoma is similar with other studies done by R. Shrikant[8] et al. Mushood G. Nabi[9] et al. and Manuneethimaram Thiygarajan[10] et al. Adedayo[11] et al. showed 71.4 % cases with < 2 cm size, which can be due to early cancer detection with regular screening programs.

In India, majority of the breast cancers are diagnosed in advanced stages which can be due to negligence in proper screening programs and lack of awareness about the signs and symptoms of the breast cancer among the people LothaR[12] et al.

In the present study, grade I tumours show 100 % positivity (3 cases) for both ER & PR, while in grade II tumours, ER & PR positivity was present in 72.7 % (8 cases) and grade III tumours does not show any ER PR positivity. While HER2 / neu positivity tumours were absent in grade I tumours, 18.18 % were positive in grade II tumours and 83.33 % positive in grade III tumours respectively. This finding in my study was comparable with the findings of RD. Puvitha[13] et al. where tumour positivity for ER & PR in grade I was 100 %, and grade II 61.36 %, and grade III tumours does not show any ER / PR positive tumours. While HER – 2 / neu positive tumours were not expressed in grade I tumours, grade II shows 56.8 % positivity and 100 % positivity in grade III tumours were observed respectively. This finding is also comparable to the study contributed by Neema Tiwari[14] et al. Javeria Iqbal[15] et al. Dr. Sumita A. Jain[16] et al. Bhagat Vasudha[7] et al. Pawan Nikhra[3] et al. &

Azizun Nisa[17] et al. where lower grades showed increased ER & PR expression and grade III tumours showed more of HER – 2 / neu expression.

KritiChauhan[18] et al. found 85.71 % of the tumour size > 5 cm has HER – 2 / neu overexpression, which is similar with the present study where tumour size > 5 cm has 80 % (4 cases) Her2 / neu over expression. Bhagat Vasudha[7] et al. & Dr. Sumita A. Jain[16] et al. also found a positive association between increasing tumour size with HER2 / neu expression showing 31.33 % & 51.1 % respectively.

Pawan Nikhra[3] et al found grade I tumours in > 60 years of age and grade II and grade III tumours were more commonly observed in 40 - 60 years of age. Similar findings were observed in the present study where 66.7 % of the grade I tumours were seen in > 60 years of age and 100 % of grade II and grade III tumours were seen in < 60 years of age. Thus, breast carcinoma occurring in older age group shows better prognosis compared to the younger age group.

In the present study ER + was seen in 55 % cases and PR + was also seen in 55 %. The study by Usha Sharma[6] et al. showed 50 % ER positivity and 47.4 % PR positivity. Pawan Nikhra[3] et al. showed 39.5 % ER positivity and 41.8 % PR positivity respectively. The HER – 2 / neu positivity in the present study were seen in 35 % of the cases. The study by Pawan Nikhra[3] et al. showed 32.5 % positivity, Bhagat Vasudha[6] et al. showed 27.58 % positivity, Lal P.[19] et al. showed 26.89 % positivity respectively in their respective studies which correlate with our study. Thus, compared to the study in western populations like Adedayo[11] et al. HER2 / neu positivity was seen more in India which may be due to lack of awareness and proper screening programs leading to diagnosis in advanced stages of breast cancer. Thus, in order to generate more statistics for future research IHC group profiling of breast cancers would be helpful.

The present study showed that HER – 2 / neu overexpression has positive association with metastatic lymph node, where 60 % of the involved nodes showed HER – 2 / neu overexpression compared to 20 % of ER & PR positive cases. Further, remaining 20 % of the node positive cases showed triple negative implying a worst outcome. This finding of the study is comparable with the studies done by KritiChauhan[18] et al. who also found 61.2 % of the nodes showed positive HER2 / neu. Study by Dr. Sumita A. Jain[16] et al. Spideh Siadati[20] et al. Aysha S. Alzaman[21] et al. also found a positive association of HER – 2 / neu with lymph node status.

In this present study increasing stage of the tumour is associated with positive HER – 2 / neu where stage I shows no HER – 2 / neu, stage 2 shows 30 % and stage III shows 57.14 % respectively. Study by Dr. Sumitha A. Jain[16] et al. found 40 %, 31.2 %, 39.9 % and 50 % HER2 / neu positivity in stage I, stage II, stage III and stage IV respectively. Cherry Bansal[22] et al. also found significant correlation between HER – 2 / neu and stage of the tumour.

Study by Neema Tiwari[14] et al. Dr Sumita A. Jain[16] et al. Bhagat Vasudha[7] et al. Mushhood[9] et al. and Lal P[19] et al. found an inverse relation between ER / PR status and HER – 2 / neu receptor which findings were similar with our findings. The present study showed two cases of papillary carcinoma of breast where both the cases showed ER & PR

positivity and negative for HER – 2 / neu implying a better prognosis. In the present study there is one case of medullary carcinoma of breast which shows triple negative hormonal status (ER / PR / HER2 / neu) implying to poorer prognosis and no response to hormonal treatment.

CONCLUSIONS

From the present study it was concluded that the most common type of breast cancer was infiltrative ductal carcinoma. Higher number of grade 1 tumours showed ER and PR positivity as compared to higher grades of tumour which are HER – 2 / neu positive. Inverse relationship was observed between ER, PR and HER – 2 / neu. HER – 2 / neu is associated with increasing size of tumour, stage and lymph node status. HER – 2 / neu status in breast cancer is important as it provides valuable prognostic, predictive and therapeutic information. HER – 2 / neu testing along with ER / PR status should be performed routinely in all patients with newly diagnosed breast cancer will guide the clinicians the correct choice of treatment protocols and also in assessing the prognosis of the patients.

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