

Original Research Article

Incidence of triple negative breast cancer at Rajendra Institute of Medical Sciences, Ranchi

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ABSTRACT

Background: Breast carcinoma is the second most common carcinoma in women and accounts for 22% of all female cancer, which is more than twice the prevalence of cancer in women at any other site. Triple negative breast cancer, i.e., negative expression of oestrogen and progesterone receptors and HER2/neu receptors and accounts for approximately 10-17% of all breast carcinomas, is biologically aggressive, resistant to conventional cytotoxic chemotherapy treatment, and is associated with reduced survival compared to other subtypes of breast cancer.

Methods: History, local examination, Various investigations like: FNAC of breast lump and axillary lymph node, USG of breasts and axilla, USG of whole abdomen and pelvis, Chest X-ray PA View etc, ER/PR and HER-2/neu status on the specimen sent for HPE were done. The prognostic implications on ER, PR and HER-2/neu receptors were assessed indirectly with the help of Nottingham prognostic index (NPI).

Results: A statistically significant correlation of ER/PR receptor status was found with tumour size, no. of lymph nodes, tumour grade and NPI, whereas HER2/neu receptor status had a statistically significant correlation with tumour size and no. of lymph node involved. Incidence of triple negative breast cancer in this Institute is 20%.

Conclusions: ER, PR and HER2/neu receptor status is highly important predictor in cases of carcinoma breast which necessitates routine evaluation of these receptor statuses for better management of disease.

Keywords: ER, PR and HER2/neu receptors, FNAC, USG, Triple negative breast cancer

INTRODUCTION

Breast carcinoma is the second most common carcinoma in women and accounts for 22% of all female cancer, which is more than twice the prevalence of cancer in women at any other site.¹ It is the most common site-specific cancer in women and is the leading cause of death from cancer for women aged 20 to 59 years.² It accounts for 26% of all newly diagnosed cancers in females and is responsible for 15% of all the cancer related death in women.³ The incidence of breast cancer has increased globally over last several decades; the greatest increase has been in Asian countries.⁴ In Asia,

breast cancer incidence peaks among women in their forties, whereas in the United States and Europe, it peaks among women in their sixties.^{5,6} Over 100,000 new breast cancer patients are estimated to be diagnosed annually in India. Breast cancer cases are expected to increase by 26% by 2020 and most of these will be seen in developing countries.⁷ Routine use of screening mammography in women >50 years of age reduces mortality from breast cancer by 33%.² In the current management guidelines, oestrogen receptor (ER), progesterone receptor (PR) status and over expression of HER2/neu are the three most useful predictive factors for response to specific therapeutic agents.⁸

Prognosis of breast carcinoma has been associated with many variables like age, tumour size, tumour grade, histological type, lymph node status, and receptor status. All above mentioned variables have got prognostic significance but receptor status has been repeatedly proved to be one of the most important prognostic factors which have effect on five- year survival rates and also mortality and disease- free survival rates.⁹⁻¹²

The ER positive /PR positive tumours appeared have more stage 1 than stage 2 tumours. Furthermore, their survival ranks from good to worse for ER positive/ PR positive to ER positive/ PR negative to ER negative/PR positive to ER negative/PR negative tumours. The ER negative/ PR negative tumours appear to have the worst prognosis, but they have the earliest age of onset and grade.¹³

Staining for oestrogen and progesterone receptors is now considered routine. Tumours are also stained for c-erb2 also known as HER2/neu (a growth factor receptor) as patients who are positive can be treated with the monoclonal antibody trastuzumab (herceptin), either in adjuvant or relapse setting¹⁴.

The immunohistochemistry (IHC) classification provides both therapeutic and prognostic information. These assays have the advantage of allowing only tumour cells to be assessed for receptor status. They can be conducted relatively inexpensively on routinely processed tissue sections with no need for spec equipment.¹⁵

Triple negative breast cancer, defined as that with negative expression of oestrogen and progesterone receptors and HER2/neu receptors and accounts for approximately 10-17% of all breast carcinomas. Triple negative breast carcinomas are associated with worst prognosis.¹⁶

Aim and objective of this Study is to study the Incidence of triple negative breast cancer at Rajendra Institute of Medical Sciences, Ranchi and correlation of hormonal receptors and HER2/neu with age, menopausal status, tumour size, lymph node status, tumour grade and NPI.

METHODS

Source of data

This study was carried out on patients with carcinoma breast admitted in the Department of General Surgery in RIMS, Ranchi from September 2015 to September 2017. Total number of 60 patients of carcinoma breast was included in the study.

Method of collection of data

Clinical data was obtained from clinical examination of patients admitted in Department of surgery. The

specimens were the tissue sent for histopathological examination (HPE) post modified radical mastectomy.

Inclusion criteria

All the cases of carcinoma breast as per fine needle aspiration cytology (FNAC) and HPE report irrespective of age who underwent surgical treatment were included in the study.

Exclusion criteria

Male patients and patients of carcinoma breast who did not undergo surgical treatment.

Parameters for assessment history and clinical examination

Each patient's detailed history was taken. Both breast and axilla were examined.

Investigations

The following tests were done on patients, i.e., FNAC of breast lump and axillary lymph node, work up for regional/distant metastasis such as USG of breast and axilla, USG of whole abdomen and pelvis, chest X-ray PA view and bone scan in indicated patients, HPE of specimen (breast tissue and lymph node removed during mastectomy) and ER/PR and HER-2/neu status on the specimen sent for H.P.E.

Analysis method

Prognostic implication on ER, PR and HER-2/neu receptors were assessed indirectly with the help of Nottingham prognostic index (NPI).

Nottingham prognostic index (NPI) = (tumour size in cm x 0.2) + lymph node stage (1=no node, 2=1 to 3 nodes, 3=4 or more nodes) + (grade 1,2,3).

RESULTS

The present study was conducted at Rajendra Institute of Medical Sciences (RIMS) Ranchi, during period of two years from September 2015 to September 2017. A total of 60 diagnosed cases of carcinoma breast were studied.

Here the patients who took part in study were from 25 to 84 years of age, maximum patients being in age group 45-54 years. The specimens from these women were sent for oestrogen, progesterone and HER2/neu receptor analysis. The mean age of the patients in the study is 49.93 years (Figure 1).

Distribution of patients according to age and ER, PR, HER2/neu status shown in Table 1.

ER, PR and HER2/neu correlated positively with increasing age but is not statistically significant ($p=0.785$, $p=0.449$ and $p=0.757$ respectively).

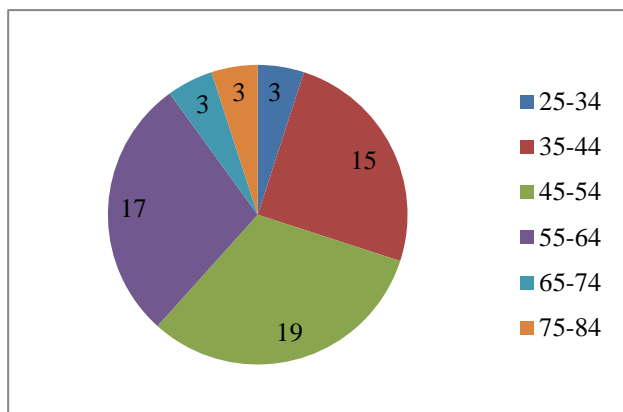


Figure 1: Age distribution of carcinoma breast patients.

Menopausal status

The patients with premenopausal status were 33 (55%) and postmenopausal were 27 (45%).

Distribution of patients according to menopausal status and ER, PR, HER/2neu status shown in Table 2.

Table 2 shows ER positivity is more common in postmenopausal women (70.37%) as compared to premenopausal women (60.60%).

Table 1: Distribution of patients according to age and ER, PR, HER2/neu status.

| AGE (yrs) | ER+ | ER- | PR+ | PR- | HER+ | HER- |
|-----------|-----|-----|-----|-----|------|------|
| 25-34 | 2 | 1 | 3 | 0 | 0 | 3 |
| 35-44 | 9 | 6 | 8 | 7 | 1 | 14 |
| 45-54 | 11 | 8 | 14 | 5 | 3 | 16 |
| 55-64 | 12 | 5 | 12 | 5 | 1 | 16 |
| 65-74 | 2 | 1 | 1 | 2 | 1 | 2 |
| 75-84 | 3 | 0 | 2 | 1 | 1 | 2 |

PR+/PR- are almost same in pre and postmenopausal women this may be due to study on small no. of patients. Menopausal status had positive correlation with ER and HER2/neu but is not statistically significant ($p=0.25$ and $p=0.676$ respectively). PR positivity and negativity were almost same in pre and postmenopausal women. Postmenopausal women had more receptor positivity for ER, HER2/neu.

Tumour size

Average size of tumour is about 5.075 cm. Size of tumour ranges from 2.5-8 cm and most of tumours were about 4-6 cm in size.

Table 2: Distribution of patients according to menopausal status and ER, PR, HER/2neu status.

| | Premenopausal | Percentage (%) | Postmenopausal | Percentage (%) |
|------|---------------|----------------|----------------|----------------|
| ER+ | 20 | 60.60 | 19 | 70.37 |
| ER- | 13 | 39.40 | 8 | 29.63 |
| PR+ | 22 | 66.67 | 18 | 66.67 |
| PR- | 11 | 33.33 | 9 | 33.33 |
| HER+ | 3 | 9.09 | 4 | 14.81 |
| HER- | 30 | 90.91 | 23 | 85.18 |

Table 3: Distribution of patients according to tumour size and ER/PR and HER2/neu status.

| Tumor size (cm) | ER+ | ER- | PR+ | PR- | HER+ | HER- |
|-----------------|-----|-----|-----|-----|------|------|
| ≤2.9 | 4 | 0 | 4 | 0 | 0 | 4 |
| 3-3.9 | 9 | 0 | 7 | 2 | 0 | 9 |
| 4-4.9 | 10 | 2 | 11 | 1 | 0 | 12 |
| 5-5.9 | 9 | 3 | 10 | 2 | 1 | 11 |
| 6-6.9 | 4 | 7 | 6 | 5 | 2 | 9 |
| 7-7.9 | 3 | 4 | 2 | 5 | 2 | 5 |
| ≥8 | 0 | 5 | 0 | 5 | 2 | 3 |

Table 4: Distribution of patients according to tumor grade and ER/PR status and HER2/neu status.

| Tumor grade | ER+ | ER- | PR+ | PR- | HER+ | HER- |
|-------------|-----|-----|-----|-----|------|------|
| W | 34 | 0 | 32 | 2 | 2 | 32 |
| M | 5 | 13 | 7 | 11 | 1 | 17 |
| P | 0 | 8 | 1 | 7 | 4 | 4 |

Distribution of patients according to tumour size and ER/PR and HER2/neu status shown in Table 3.

Table 3 shows as tumour size increases ER/PR negativity and HER2/neu positivity increases. HER2/neu positivity is seen with tumour size >5.

Tumour size had inverse correlation with ER and PR positivity which is highly statistically significant (p=0.001), and had positive correlation with HER2/neu positivity which is statistically significant (p=0.042).

Tumour grade

Tumours were graded as well differentiated (W), moderately differentiated (M), and poorly differentiated (P). Most of patients belongs to well differentiated group (34) followed by moderately differentiated (14) and poorly differentiated (12).

Distribution of patients according to tumor grade and ER/PR status and HER2/neu status shown in Table 4.

Table 4 shows ER/PR positive and HER2/neu negative tumours are well differentiated (grade1) in contrast ER/PR negative and HER2/neu positive tumour are poorly differentiated (grade 3).

Histological grade of tumour had an inverse correlation with ER and PR positivity which is highly significant statistically (p=0.000), and had positive correlation with HER2/neu positivity which is not statistically significant (p=0.105).

Tumour histology

In 56 (93.33%) patients, histological type was infiltrating ductal carcinoma (IDC) and in rest 4 (6.67%) Mucinous carcinoma.

Lymph node

Total number of positive axillary lymph nodes ranges from 0-7 (mean=2.4).

Distribution of patients according to lymph nodes and ER, PR and HER2/neu status shown in Table 5.

Table 5: Distribution of patients according to lymph nodes and ER, PR and HER2/neu status.

| No. of lymph nodes | ER+ | ER- | PR+ | PR- | HER+ | HER- |
|--------------------|-----|-----|-----|-----|------|------|
| 0 | 12 | 0 | 11 | 1 | 0 | 12 |
| 1 | 12 | 2 | 12 | 2 | 0 | 14 |
| 2 | 9 | 3 | 9 | 3 | 0 | 12 |
| 3 | 3 | 4 | 4 | 3 | 0 | 7 |
| 4 | 1 | 4 | 2 | 3 | 1 | 4 |
| 5 | 2 | 2 | 2 | 2 | 2 | 2 |
| 6 | 0 | 4 | 0 | 4 | 2 | 2 |
| 7 | 0 | 2 | 0 | 2 | 2 | 0 |

Table 6: Distribution of patients according to NPI and ER, PR and HER2/neu status.

| NPI | ER+ | ER- | PR+ | PR- | HER+ | HER- |
|---------|-----|-----|-----|-----|------|------|
| ≤2.4 | 1 | 0 | 1 | 0 | 0 | 1 |
| 2.5-3.4 | 10 | 0 | 10 | 0 | 0 | 10 |
| 3.5-4.4 | 22 | 0 | 20 | 2 | 0 | 22 |
| 4.5-5.4 | 5 | 4 | 6 | 3 | 2 | 7 |
| ≥5.5 | 1 | 17 | 3 | 15 | 5 | 13 |

Table 7: Distribution of patients according to ER, PR, HER2/neu status and number of cases.

| Immunohistochemistry result | No. of cases |
|---------------------------------------|--------------|
| (ER/PR)+, HER2/neu- | 33 |
| (ER/PR)+, HER2/neu+ | 2 |
| (ER/PR)-, HER2/neu+ | 4 |
| (ER/PR)-, HER2/neu- (triple negative) | 12 |
| ER+, PR-, HER2/neu+ | 1 |
| ER+, PR-, HER2/neu- | 3 |
| ER-, PR+, HER2/neu+ | 0 |
| ER-, PR+, HER2/neu- | 5 |

Table 5 shows ER/PR positivity is associated with decreased lymph node involvement and HER2/neu negativity is associated with decreased lymph node involvement. In contrast ER/PR negativity and HER2/neu positivity associated with increased lymph node involvement.

Lymph node number had an inverse correlation with ER and PR positivity which is highly statistically significant ($p=0.000$ and $p=0.005$ respectively), and had positive correlation with HER2/neu positivity which is highly statistically significant ($p=0.000$).

Nottingham prognostic index

Nottingham prognostic index (NPI) is calculated with the help of following formula.

$NPI = (\text{Tumour size in cm} \times 0.2) + \text{Lymph node stage}$
(1=no node, 2 = 1-3 nodes, 3= 4 or more nodes) + Grades (1, 2 or 3).

Distribution of patients according to NPI and ER, PR and HER2/neu status shown in Table 6. Table 6 shows ER/PR negativity and HER2/neu positivity is associated with increased NPI i.e., poor prognosis.

NPI value had inverse correlation with ER and PR positivity which is highly significant statistically ($p=0.000$), and had positive correlation with HER2/neu positivity which is not statistically significant ($p=0.142$).

Distribution of patients according to ER, PR and HER2/neu status

Distribution of patients according to ER positive status were 39 (65%), ER negative were 21 (35%), for PR positive 40 (66.67%), PR negative were 20 (33.33%), HER2/neu positive patients were 7 (11.67%) and HER2/neu negative were 53 (88.33%),

Distribution of patients according to ER, PR, HER2/neu status and number of cases shown in Table 7.

Among 60 patients triple negative breast cancer cases were found in 12 (20%) and non-triple negative breast cancer cases were 48 (80%).

DISCUSSION

The present study was conducted at Rajendra Institute of Medical Sciences (RIMS) Ranchi, during period of two years from September 2015 to September 2017. A total of 60 diagnosed cases of carcinoma breast were studied.

Age distribution among patients with carcinoma breast

Result in present study (Table 1A and B), out of 60 patients of carcinoma breast who were from 25 to 84

years of age, ER+ and PR+ cases were 39 (65%) and 40 (66.67%) and, ER, PR- were 21 (35%) and 20 (33%) respectively. Mean age was 49.93. p value for ER/PR/HER2/neu with age was not statistically significant ($p=0.785$, $p=0.757$, $p=0.449$ respectively). Still, as the age increased ER/PR positivity increased which is in accordance with below studies. This is probably related to age related hormonal changes in body. Fisher et al studied 178 invasive breast cancer cases. Well differentiated tumours were more frequently ER+ in older women.¹⁷ Ruder et al in 1989 reported a study over 171 Israeli women diagnosed with breast cancer among which 77 ER- versus 94 ER+ and 69PR- versus 65PR+ were present. Age tended to be associated positively with both ER+ and PR+.¹⁸

Result in present study showed that out of 60 patients 7 patients were HER 2/neu + and among them 3 were of age <50, 4 were of age >50. A study by Al- Moundhri showed where 12 out of 13 patients with positivity for HER2/neu were >40 years of age. However many other studies showed increased HER2/neu expression in younger age group Saleh et al and Ambroise et al.^{20,21} Studies by Huang et al, Rashed et al and Vaidyanathan et al showed no correlation of HER2/neu expression with age.²²⁻²⁴

Menopausal status and ER, PR, HER2/neu receptor

Result in present study showed that ER positivity common in postmenopausal women (70.37%) as compared to premenopausal women (60.60%) and ER negativity is more common in premenopausal women (39.40%) as compared to (29.63%) in postmenopausal women. PR+/PR- is same in pre and postmenopausal women this may be due small number of patients included in the study. Result is in accordance with Mohla et al for ER and in accordance with Eisenberg et al for PR however no statistically significant association found between ER/PR and HER2/neu with menopause ($p=0.25$, $p=0.713$, $p=0.676$ respectively). Mohla et al in 1982 studied 146 black women with breast cancer and found that postmenopausal patients with primary tumours showed higher ER positivity than premenopausal patients and metastatic sites, respectively.^{25,26} Eisenberg et al in 2001 found a statistically significant positive association between ER and menopausal status (pre- vs. post-menopause), ($p=0.0008$). The association observed between PR and this same variable was small and not statistically significant ($p=0.37$).²⁶

Patients in postmenopausal age group showed more HER2/neu positivity (14.81%) than premenopausal age group (9.09%) in this study. No statistically significant association of HER2/neu with menopausal status was noted in present study ($p=0.676$). Similarly, studies by Rashed et al and Vaidyanathan et al showed not statistically significant correlation of HER2/neu with menopausal status.^{23,24}

Tumour size and ER, PR, HER2/neu receptors

In the present study tumour size ranges from 2.5- 8 cm, with average tumour size was 5.075 cm. Most of tumour was 4-6 cm in size. Tumour size has inverse correlation with ER, PR positivity which is statistically significant (p value=0.001). As the tumour size increased, the ER and PR negativity has increased which in accordance with below studies. Eisenberg found statistically significant association between ER and PR positive tumours and tumour size less than 4.0 cm (p<0.005).²⁶ Goyanes found that oestrogen receptor (ER) expression was associated with low nuclear grade and histological grade, and with smaller tumour size (p<0.05).²⁷

In present study while correlating HER2/neu positivity with the size of tumour, majority was seen with tumour size >5 cm. As the tumour size increased HER2/neu positivity increased which is found to be statistically significant (p=0.042).

Lymph node number and ER, PR, HER2/neu receptors

In the present study number of axillary lymph node involved was 0-7. A statistically significant association was found between ER, PR receptor and lymph node status (p=0.000, p=0.005 respectively). More number of lymph node involvements was associated with negative ER, PR status. Stieret et al and Grogan et al showed that presence of hormonal receptors (ER and PR) were not associated with nodal status.^{30,31} Eisenberg et al did not find any association of nodal status with hormonal receptors.²⁶ Goyanes found that ER expression tended to decrease as the number of metastatic axillary lymph nodes increased, although association was not statistically significant.²⁷ Ahmed et al found a significant positive association between ER or PR expression with lymph node involvement (p=0.000, p=0.022 respectively).³⁰

In this study as HER2/ neu positivity increased, number of lymph node involved increased which was found to be statistically significant (p=0.000) which was found in accordance with below studies. In the studies by Tatjana et al, Moradi et al, Nisa et al and Naeem et al also showed increased expression of HER2/neu in cases of lymph node involvement and only study Azizun- Nisa had a statistically significant correlation.³¹⁻³⁵

Histological grade and ER, PR, HER2/neu receptor status

In the present study most of tumour were well differentiated (W)-34, followed by moderately differentiated (M)-14 and poorly differentiated (P)12. Tumour grade had an inverse correlation with ER/PR positivity which was statistically significant (p=0.000). The poorly differentiated tumours are less likely to express ER/PR which was in accordance with below studies. Fisher et al found positive ER to be significantly associated with high nuclear grade and low histologic

grades.¹⁷ Mohla et al also found a significant correlation between the ER positivity and tumour grade.²⁵ Eisenberg et al found statistically significant association between ER and PR positive tumour and low histological grade (p=0.01).²⁶ Goyanes et al also found significant association between ER and PR positive tumours and low histological grade (p=0.01).²⁷

In this study HER2/neu positivity is associated with higher histological grade II, III. Studies by various authors showed similar result. No statistically significant association of HER2/neu with histological grade was noted in present study (p=0.105) similar to the studies by Naeem and Dogan.^{28,29} Unlike our study, most of the studies by Saleh et al, Moradi et al and Nisa et al showed statistically significant association of HER2/neu with histological grade.^{20,34,35}

NPI and ER, PR, HER2/neu receptor status

Prognostic implication of ER/PR receptors were assessed indirectly with the help of Nottingham Prognostic Index (NPI). This index predicts the survival of patients with invasive breast cancer, and it can define 3 subsets of patients with different chances of dying from breast cancer; good (≤ 3.4), moderate (3.41-5.4), and poor (> 5.4) prognostic groups.³⁶ In the present study ER/PR positivity has a statistically significant correlation with NPI; higher the NPI, the positivity of both ER and PR status decreases (p=0.000). Hence ER/PR positive receptor implies good prognosis. And ER/PR negativity was associated with higher value of NPI.

In this study higher value NPI was associated with HER2/neu positivity, i.e., poor prognostic group (III). However no studies correlating NPI with HER2/neu with best of our knowledge has been done so far. In the present study, association of HER2/neu with NPI was not statistically significant (p=0.142).

Incidence of triple negative breast carcinoma

In the present study out of 60 patients, 33 (55%) were ER+/PR+, HER2/neu- and triple negative carcinomas were 12 (20%). Similar observations were seen in Onitilo et al and Huang et al.^{37,38} Non triple negative breast carcinomas were 48 (80%). Hence incidence of triple negative breast carcinoma in this institute is 20%.

CONCLUSION

This study at Rajendra Institute of Medical Sciences (RIMS) Ranchi evaluated incidence of triple negative breast cancer at this institute and correlated ER, PR and HER2/neu with various prognostic factors.

According to the data of this study, a statistically significant correlation of ER/PR receptor status was found with tumour size, no. of lymph nodes, tumour grade and NPI, whereas HER2/neu receptor status had a

statistically significant correlation with tumour size and no. of lymph node involved.

To conclude ER, PR and HER2/neu receptor status is highly important predictor in cases of carcinoma breast which necessitates routine evaluation of these receptor statuses for better management of disease.

Triple negative breast cancer (TNBC) is associated with poor prognosis. Hence, immunohistochemical assessment of ER, PR and HER2/neu should be incorporated as a routine investigation.

Incidence of triple negative breast cancer in this institute is 20% which is slightly higher as compared to previous studies. This high incidence may be due to unawareness of patients about carcinoma breast, testing of hormonal and HER2/neu receptors and its importance for treatment.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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