STUDY OF EXPRESSION OF VARIOUS IMMUNOHISTOCHEMICAL MARKERS IN THE INVASIVE DUCTAL CARCINOMA OF BREAST (IDC).

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ABSTRACT:

INTRODUCTION:

Breast cancer is the most frequent cancer in women worldwide, affecting one out of every eight women.[1] It accounts for 25% of all invasive cancers in females and 16% of all female malignancies.[2] The World Health Organization (WHO) reports that 685,000 women died from breast cancer in 2020, with an estimated 2.3 million women receiving a diagnosis worldwide. Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life. [3]

If detected at an early stage, breast cancer is curable. Prognostic factors are those that affect a patient's overall prognosis, such as the likelihood of recurrence following treatment. These variables aid in choosing the right patients for a certain treatment. [6] Tumor size, tumour grade, and axillary lymph node metastases are examples

of conventional morphological prognostic variables. As a result of the fact that a considerable proportion of patients with early-stage breast cancer already have microscopic metastases when they are diagnosed, biological molecular prognostic markers are being given more weight.[7] The most relevant clinical biomarkers that are widely used in managing breast cancer cases according to stage are hormone receptors (ER and PR) and human epidermal growth factor receptor-2 (HER-2).[7] Making management decisions and plans for breast cancer management requires an understanding of hormone receptors and HER-2 expressions. [8] In treatment of breast cancer, prognostic and predictive factors are used. Predictive factors assess the likelihood of benefit from a certain treatment. ER, PR, and HER2/neu are prognostic as well as predictive variables. [6]

METHOD: This present study, entitled "IMMUNOHISTOCHEMICAL (IHC) EXPRESSION OF ESTROGEN RECEPTOR (ER), PROGESTERONE RECEPTOR (PR) AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR-2 (HER2/NEU) IN INVASIVE DUCTAL CARCINOMA OF BREAST: A CLINICOPATHOLOGICAL STUDY" is conducted in the Department of Pathology at Dhiraj hospital from 1st February 2023 to 31st August 2023.

- The recorded data of IHC of patients who were diagnosed as invasive ductal carcinoma of breast that received in the department of pathology at Dhiraj hospital from 1st February 2023 to 31st August 2023.

RESULT: The present study was undertaken to evaluate the various IHC markers expression in invasive ductal carcinoma of breast. Total of sixty cases who met the inclusion criteria were taken into the study.

CONCLUSION: The present study reported maximum number of cases in the age group of 51–60 years. Majority of cases were in grade II followed by grade III and grade I. The highest proportion of positive ER (18.33%), and PR (16.67%) expressions were observed in the age group 51-60 years. The highest proportion of positive HER/2neu (10%) expression was observed in the age group 51-60 & 61-70 years.

KEY WORDS: ER, PR, HER/2neu

INTRODUCTION

Breast cancer is the most frequent cancer in women worldwide, affecting one out of every eight women.[1] It accounts for 25% of all invasive cancers in females and 16% of all female malignancies. [2] The World Health Organization (WHO) reports that 685,000 women died from breast cancer in 2020, with an estimated 2.3 million women receiving a diagnosis worldwide. Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life. [3]

The most prevalent malignant tumor and the main contributor to cancer-related fatalities in women is breast carcinoma. In industrialized nations, it is more prevalent.[4] Although there are many different forms of breast carcinomas, infiltrating ductal carcinoma is the most prevalent histological type. [5] The mainstay of breast cancer treatment is surgery when the tumour is confined, followed by chemotherapy for cancers that are

positive for the estrogen receptor (ER) and progesterone receptor (PR), adjuvant hormonal therapy, and radiotherapy.

If detected at an early stage, breast cancer is curable. Prognostic factors are those that affect a patient's overall prognosis, such as the likelihood of recurrence following treatment. These variables aid in choosing the right patients for a certain treatment. [6] Tumor size, tumour grade, and axillary lymph node metastases are examples of conventional morphological prognostic variables. As a result of the fact that a considerable proportion of patients with early-stage breast cancer already have microscopic metastases when they are diagnosed, biological molecular prognostic markers are being given more weight.[7] The most relevant clinical biomarkers that are widely used in managing breast cancer cases according to stage are hormone receptors (ER and PR) and human epidermal growth factor receptor-2 (HER-2).[7] Making management decisions and plans for breast cancer management requires an understanding of hormone receptors and HER-2 expressions. [8] In treatment of breast cancer, prognostic and predictive factors are used. Predictive factors assess the likelihood of benefit from a certain treatment. ER, PR, and HER2/neu are prognostic as well as predictive variables. [6]

Estrogen receptor (ER):

During a woman's lifetime, the breasts experience significant physiological changes, and estrogen actively mediates these changes. ER comes in two varieties: ERα and ERβ. [9] The receptor ERα is a well-established prognostic and predictive factor in breast cancer.   It is unclear what role ERβ plays in prognosis.[9],[10] The majority of ER-positive breast tumours comprise both ERα and ERβ subtypes, however some tumours only express ERβ. Different clinical responses and behaviours may result from this. Contrary to ERα, ERβ expression declines during breast carcinogenesis.

Progesterone receptor (PR):

There are two different types of PR: PR-A and PR-B. The function of estrogen is modulated by progesterone. It has been found that ER-positive breast tumours without PR expression respond to hormone therapy less than those with PR expression. It is also evident that the phenotypes of ER and PR are not constant. These may alter as the disease progresses naturally or as a result of medical intervention.

Human epidermal growth factor receptor-2/neu (c-erbB-2):

It is one of the four members family of the closely related growth factor receptors, which includes HER1, HER2, HER3, and HER4. Breast cancer oncogenic transition and carcinogenesis both entail HER2/neu amplification or overexpression. Overexpression of the receptor leads to unnecessarily enhanced signalling. It may cause uncontrolled cell proliferation to increase, apoptosis to decline, cancer cell motility to increase, angiogenesis to increase, and a worse prognosis as a result.

As a result of the survival benefit associated with adjuvant hormonal or chemotherapeutic therapies for patients with hormone receptor positive status, ER, PR, and HER2/neu receptor status determination in breast cancer has currently become a frequent practice. Strong ER-positive cases, as opposed to those with low to moderate ER positivity, benefit from endocrine therapy alone, as is well documented.

AIM AND OBJECTIVES

AIM

- To study the expression of various immunohistochemical markers in the Invasive Ductal Carcinoma of breast (IDC).

OBJECTIVES

- To explore distribution of various IHC markers in IDC.

To study the correlation of IHC markers to various clinicopathological parameters: Age of the patient, histological grade, tumor size and lymph node metastasis.

MATERIALS AND METHODS

This present study, entitled "IMMUNOHISTOCHEMICAL (IHC) EXPRESSION OF ESTROGEN RECEPTOR (ER), PROGESTERONE RECEPTOR (PR) AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR-2 (HER2/NEU) IN INVASIVE DUCTAL CARCINOMA OF BREAST: A CLINICOPATHOLOGICAL STUDY" is conducted in the Department of Pathology at Dhiraj Hospital from 1st February 2023 to 31st August 2023.

- ➢ **SOURCE OF DATA:**
  - The recorded data of IHC of patients who were diagnosed as invasive ductal carcinoma of breast that received in the department of pathology, Dhiraj Hospital during period of 1st February 2023 to 31st August 2023.

- ➢ **INCLUSION CRITERIA**:
  - All invasive ductal carcinoma of breast diagnosed in MRM specimens at central lab in Dhiraj Hospital.

- ➢ **EXCLUSION CRITERIA**:
  - All benign tumors and inflammatory lesions.
  - Malignant stromal tumors.
  - Lobular carcinoma.

Statistical analysis: Data collected was analysed statistically using percentage and frequency distribution and was presented in the form of tables, charts and graphs.

RESULTS AND OBSERVATIONS

The present study was undertaken to correlate the expression of ER, PR, and HER2/neu with each other and to various clinicopathological parameters such as: age of the patient, histological grade, tumor size, and lymph node metastasis in 60 cases of invasive ductal carcinoma, breast in the department of Pathology, Dhiraj Hospital, Waghodia.

Age prevalence:

In the present study, a total of 60 cases were studied. They were divided into six age groups, first group included women of 21-30 years, second group 31-40 years women, third one included women of 41-50 years, fourth group included women of 51-60 years, fifth group included women of 61-70 years and sixth group included women of 71-80 years. In our study lowest age was 28 years & the highest age was 76 years with the mean age of 52.6 years.

Maximum number of cases (33.33%) were in the age group of 51-60 years. (Graph 1)
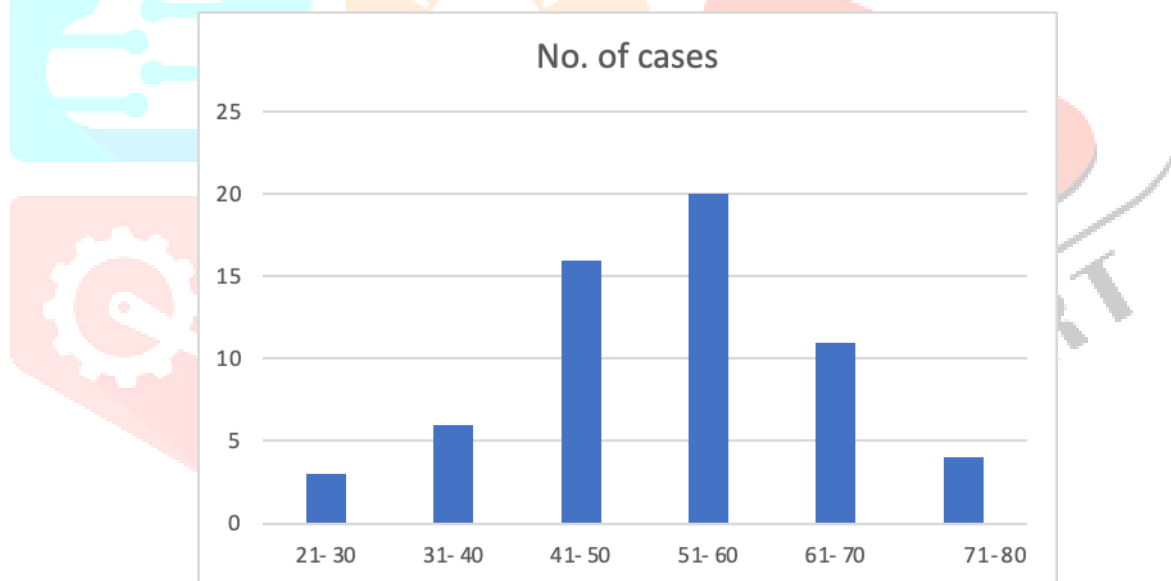
Graph 1: Distribution of the cases based on age.

Distribution of carcinoma cases according to the grade of the tumor:

In the present study, grading was done according to Nottingham modification of Bloom Richardson grading system. We found 7 cases of grade I, 28 cases of grade II & 25 cases of grade III. (Table 1)

Tumor grade	No. of cases (n=60)
Grade I	07(11.66%)
Grade II	28(46.67%)
Grade III	25(41.67%)

Table 1: Distribution of carcinoma according to the grade of the tumor

Lymph node status of tumor:

Out of 60 cases, 34 cases were lymph node positive, and 26 cases were lymph node negative. (Table 2)

Lymph node	No. of cases
Positive	34(56.67%)
Negative	26(43.33%)
Total	60

Table 2: Lymph node status of tumor

Immunohistochemical expression:

In present study, total number of 60 cases were studied for expression of Estrogen receptor (ER), progesterone receptor (PR) and HER2/neu in invasive ductal carcinoma, breast. Out of which 45% were ER+/PR+/HER2-, 23.33% were ER-/PR-/HER2-, 21.67% were ER-/PR-/HER+, 5% were ER+/PR+/HER2+, 3.33% were ER+/PR-/HER2-, 1.67% were ER+/PR-/HER2+. ER-/PR+/HER2- & ER-/PR+/HER2+ seen in zero cases. (Table 3)

Hormone receptor	Number	Percentage (%)
ER+/PR+/HER2-	27	45
ER-/PR-/HER2-	14	23.33
ER-/PR-/HER+	13	21.67
ER+/PR+/HER2+	3	5
ER+/PR-/HER2-	2	3.33
ER+/PR-/HER2+	1	1.67
ER-/PR+/HER2-	0	0
ER-/PR+/HER2+	0	0
Total	60	100%

Table 3: Expression of ER, PR & HER2/neu in IDC

ER, PR AND HER2/neu:

Out of 60 cases, Estrogen Receptor (ER) positivity was noted in 33 (55%) cases, Progesterone Receptor (PR) positivity was noted in 30 (50%) cases and HER2/neu positivity in 17 (28.33%) cases. (Table 4)

	ER	PR	HER2/neu
Positive	33 (55%)	30 (50%)	17 (28.33%)
Negative	27 (45%)	30 (50%)	43 (71.67%)
Total	60	60	60

Table 4: Frequency of ER, PR and HER2/neu in IDC

ER & PR:

Out of 60 cases, 30 cases were ER and PR positive, 27 cases were ER & PR negative, and 3 cases showed different expressions of ER & PR. (Table 5)

ER	PR positive	PR negative	Total
Positive	30	03	33
Negative	00	27	27
Total	30	30	60
χ^2 , df, P	49.09, 1, 0.000		

Table 5: Expression of ER & PR in IDC

There was significant correlation between the ER & PR ($\chi^2 = 49.09$, p = 0.000).

ER & HER2/neu:

Out of 60 cases, 4 cases were ER & HER2/neu positive, 14 cases were ER & HER2/neu negative and 42 cases showed different expression of ER & HER2/neu. (Table 6)

ER	HER2/neu positive	HER2/neu negative	Total
Positive	04	29	33
Negative	13	14	27
Total	17	43	60
χ^2, df, P	9.49, 1, 0.002		

Table 6: Expression of ER & HER2/neu in IDC

There was significant correlation between the ER & HER2/neu ($\chi^2 = 9.49$, p = 0.002).

PR & HER2/neu:

Out of 60 cases, 3 cases were PR & HER2/neu positive, 16 cases were PR & HER2/neu negative and 41 cases showed different expression of PR & HER2/neu. (Table 7)

PR	HER2/neu positive	HER2/neu negative	Total
Positive	03	27	30
Negative	14	16	30
Total	17	43	60
χ^2, df, P	9.93, 1, 0.001		

Table 7: Expression of PR & HER2/neu in IDC

There was significant correlation between the PR & HER2/neu ($\chi^2 = 9.93$, p = 0.001).

IHC and age distribution:

Proportion of cases showing positive reactivity for ER and PR hormone receptor was higher in 51- 60 years of age group (18.33% & 16.67% respectively) and for Her2/neu hormone receptor was higher in the age group of 51- 60 & 61-70 years (10%). (Table 8)

Age in range(in years)	ER			PR			HER2/neu		
	positive	negative	total	positive	negative	total	positive	negative	total
21- 30	1	2	3	1	2	3	1	2	3
31- 40	5	1	6	5	1	6	0	6	6
41- 50	7	9	16	6	10	16	4	12	16
51- 60	11	9	20	10	10	20	6	14	20
61- 70	5	6	11	4	7	11	6	5	11
71- 80	4	0	4	4	0	4	0	4	4
Total	33	27	60	30	30	60	17	43	60
χ^2, df, P	7.01, 5, 0.219			8.81, 5, 0.116			7.82, 5, 0.166		

Table 8: ER, PR, HER2/neu & Age

IHC and size of the tumor:

Majority of ER/PR and HER2/neu positive tumors were of size between 2 and 5 cm (40%, 36.67% & 21.67% respectively). (Table 9)

Size of tumor (cm)	ER			PR			HER2/neu		
	positive	negative	total	positive	Negative	total	positive	negative	total
<2	1	2	3	0	3	3	0	3	3
2-5	24	20	44	22	22	44	13	31	44
>5	8	5	13	8	5	13	4	9	13
Total	33	27	60	30	30	60	17	43	60
χ^2, df,P	0.79, 2, 0.671			3.69, 2, 0.157			1.25, 2, 0.533		

Table 9: ER, PR, HER2/neu & size of tumor

There was no significant correlation between the IHC markers & size of the tumor (ER & tumor size : χ^2 = 0.79, p = 0.671, PR & tumor size: χ^2 = 3.69, p = 0.157, HER2/neu & tumor size: χ^2 = 1.25, p = 0.533).

IHC and tumor grade:

Majority of ER/PR and HER2/neu positive tumors were of grade II (30%, 26.67% & 15% respectively). (Table 10)

Tumor grade	ER			PR			HER2/neu		
	positive	negative	total	positive	negative	total	positive	negative	total
Grade I	6	1	7	6	1	7	1	6	7
Grade II	18	10	28	16	12	28	9	19	28
Grade III	9	16	25	8	17	25	7	18	25
Total	33	27	60	30	30	60	17	43	60
$\chi2$, df, P	7.29, 2, 0.026			7.38, 2, 0.024			0.88, 2, 0.643		

Table 10: ER, PR, HER2/neu & tumor grade

There was significant inverse correlation between the hormone receptors (ER & PR) & tumor grade (ER & tumor grade: $\chi2 = 7.29$, p = 0.026, PR & tumor grade: $\chi2 = 7.38$, p = 0.024), whereas there was no significant correlation between the HER2/neu & tumor grade ($\chi2 = 0.88$, p = 0.643).

IHC and lymph node status of tumor:

All cases were evaluated for lymph nodes metastasis and found that 34 cases had lymph nodes metastasis. Out of 33 ER positive cases, 20 had positive lymph nodes, whereas out of 30 PR positive cases, 17 had positive lymph nodes and out of 17 HER2/neu positive cases, 12 had positive lymph nodes. (Table 11)

Lymph node	ER			PR			HER2/neu		
	positive	negative	total	positive	negative	total	positive	negative	total
Positive	20	14	34	17	17	34	12	22	34
Negative	13	13	26	13	13	26	05	21	26
Total	33	27	60	30	30	60	17	43	60
$\chi2$, df, P	0.46, 1, 0.496			0, 1, 1			1.87, 1, 0.171		

Table 11: ER, PR, HER2/neu & LN status of tumor

There was no significant correlation between the IHC markers & LN status of the tumor (ER & LN: $\chi2 = 0.46$, p = 0.496, PR & LN: $\chi2 = 0$, p = 1, HER2/neu & LN: $\chi2 = 1.87$, p = 0.171).

SUMMARY AND CONCLUSION:

The present study was undertaken to study the expression of hormone receptors ER / PR & HER2/neu status by immunohistochemistry in 60 cases of invasive ductal carcinoma of breast in the Dhiraj Hospital, Department of Pathology, Waghodia.

1. The present study reported maximum number of cases in the age group of 51–60 years (33.33%) with the mean age of 52.6 years.

2. The tumor size varied from 0.8 to 13 cm. Majority of tumors were in the size range of 2 to 5cm (73.33 %).

3. In the present study, majority of cases (46.67%) were in grade II followed by 41.67% in grade III and grade I (11.66%).

4. In the present study, 34 cases (56.67%) were lymph node positive, and 26 (43.33%) cases were lymph node negative.

5. In present study, ER & PR were positive in 33 (55%) & 30 (50%) cases, respectively.

6. Over expression of HER2/neu was observed in 17 (28.33%) cases. Negative expression of HER2/neu is higher than positive expression.

7. Majority of cases were ER+/PR+/HER2- (45%). The present study reported high proportion of hormone receptors ER / PR positive tumors (50%).

8. In our study, ER and PR were positively correlated with each other (P=0.000), whereas a statistically significant inverse relationship was obtained between HER-2/neu and ER (p=0.002) and HER-2/neu and PR (p=0.001).

9. The highest proportion of positive ER (18.33%), and PR (16.67%) expressions were observed in the age group 51-60 years. The highest proportion of positive HER/2neu (10%) expression was observed in the age group 51-60 & 61-70 years. There was no statistically significant association between age of the patient and ER, PR and HER2/neu expression (P=0.22, P= 0.12 & P=0.17 respectively) in our study.

10. In our study, majority of (40%) ER positive, 36.67% PR positive and 21.67% HER2/neu positive cases were in the size range of 2 to 5 cm. We have found that as the tumor size increases, ER, PR & HER2/neu positive expression decreases. We did not find any correlation between size of tumor and ER, PR & HER2/neu (P= 0.67, P=0.15, P= 0.53 respectively).

11. In present study, majority of ER, PR and HER2/neu positive cases (30%, 26.67% & 15% respectively) were of grade II. Expression of hormone receptors ER / PR had an inverse co-relation with the Nottingham modification of Bloom Richardson grade (P=0.026 & P=0.024 respectively). Whereas, Her2/neu expression did not reveal any significant association with tumor grade (P=0.64).

12. ER, PR & HER2/neu positivity were higher in node positive cases than node negative cases. There was not any significant association between ER, PR & HER2/neu and lymph node status (P=0.5, P=1 & P=0.17 respectively).

13. Finally, immunohistochemistry is a good technique to test expression of hormone receptors ER / PR & over expression of HER2/neu in breast tumors. It should be advocated in each & every breast tumor to detect expression of hormone receptors ER / PR & HER2/neu as hormone therapy in addition to surgery can reduce the chances of recurrence & increase the survival of patient.

14. The IHC classification provides both therapeutic and prognostic information.

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