JCOMS eISSN:2783-3127 https://journalofcoms.com

Journal of

Current Oncology and Medical Sciences

Vol. 4, No.1

Original Free Access

A histopathological study on breast carcinoma with special reference to cyclin-D1 and estrogen receptor

Payel Hazari [1], Monoj Kumar Deka [1], Bandana Kanoo [1]

[1] Department of Pathology, Silchar Medical College and Hospital, India

Abstract

Introduction: Breast cancer is the most frequent cause of cancer-related death in women in developing nations. Breast cancer diagnoses have increased as a result of rising awareness among women. The expression of Estrogen receptors (ER) plays a crucial role in determining the responsiveness to specific treatments. Cyclin D1 being a marker for cell proliferation was used in this study. The primary objectives of the current investigation were to investigate the expression of Cyclin-D1 and Estrogen receptor (ER) in breast carcinoma and to establish a relationship between the expression patterns of Cyclin-D1 and ER with the histopathological features of the tumor in breast carcinoma.

Materials and methods: The study was conducted in the Department of Pathology, Silchar Medical College and Hospital, Silchar, India, from June 2021 to May 2022. A total of 59 cases of primary breast carcinoma MRM(Modified radical mastectomy) specimens were included in the study.

Results: The mean age of the patients was 52.12 ± 12.47 years, and the majority of the patients were in the post-menopausal phase. Lymph node metastasis was observed in 47.5% of the cases, and the majority of the cases were in grade II. The study demonstrated a trend towards increased Cyclin-D1 and ER-positive with aging. Cyclin-D1 positivity decreases and Cyclin-D1 negativity increases as the tumor growth increases. The study showed a statistically significant association (P=0.001) between ER and Cyclin-D1. The majority of post-menopausal patients had ER-positive.

Conclusion: The present study provides the incidence of different parameters associated with breast carcinoma and their statistical correlation with CyclinD1 and ER that will provide improved and crucial treatment guidance.

Keywords: Breast carcinoma, Histopathological grades, Lymph node metastasis, Estrogen receptor (ER), Cyclin-D1, Menopausal status

Corresponding Authors: Payel Hazari

✉ Email: hazari.payel2@gmail.com

Receive: 2024.1.14, Accepted: 2024.3.30

Introduction

Breast carcinoma accounts for 1 in 4 cancer diagnoses among women worldwide. Breast cancer, which accounts for an anticipated 2,261,419 cases (11.7% of all cancer sites) each year (2020) and 684,996 fatalities (6% of all cancer-related deaths), is the most common worldwide (2020). Breast cancer is the most frequent cause of cancer-related death in women in developing nations. In India, 178 361 (26.3%) new cases were found among the female population in 2020 (1). Breast cancer diagnoses have increased as a result of rising awareness among women.

Breast cancer has a high rate of survival when detected early and when there is access to effective therapy. Unfortunately, 50–80% of these illnesses are found at an advanced stage in the majority of low- and middle-income countries (2). A more sensitive assessment of a palpable breast lump has recently been employed with the help of the Triple Test approach, which consists of a clinical examination, mammography, and fine needle aspiration cytology (3). Early diagnoses of aggressive tumors (ER-ve, PR-ve, HER2/neu +ve, or triple-negative tumors) result from increased awareness campaigns (4, 5, 6).

As per the latest treatment guidelines for breast cancer (7), the expression of estrogen receptors (ER) plays a crucial role in determining the responsiveness to specific treatments. The ER expressions are critical in determining how well hormonal therapy will function (8).

Histopathologists commonly assess tumor proliferation activity, which provides data on the clinical behavior, diagnosis, and treatment of tumors (9). Cyclins bind to and activate Cyclin-Dependent Kinases (CDK), regulating the rate at which cells transition between different cell cycle phases. In this study, cyclin D1 was used as a marker for cell proliferation.

Cyclin D1 activates steroid hormone receptor-mediated transcription in the absence of estrogen hormone and enhances transcription in its presence. The anti-estrogens did not inhibit the activation of estrogen receptors by Cyclin D1.There is an increase in binding of the receptor to estrogen response element sequence that upregulates ER-mediated transcription owing to the direct binding of Cyclin-D1 to the hormone binding domain of ER. These results highlight a unique role for Cyclin D1 as a CDK-independent matter of the ER (10).

This study aims to investigate various parameters like age, laterality, menopausal status, tumour size, lymph node and the expression of ER and Cyclin-D1 in breast cancer and to establish a relationship between the expression patterns of Cyclin-D1 and ER with the histopathological features of the tumor in breast carcinoma. This will provide improved and crucial treatment guidance for breast cancer patients.

Materials and Methods

The present study was undertaken to study the clinic-pathological findings in breast carcinoma and to assess the expression of Cyclin-D1 and ER in them.

Place of study

The present study was undertaken in the Department of Pathology, Silchar Medical College and Hospital, Silchar. The study was approved by the Institute's Ethics Committee (No. SMC/15,222) dated 20/10/2022. According to the Helsinki Declaration's ethical guidelines, the study is compliant.

Study period

1 year: From June 2021 to May 2022.

Type of Study

Hospital-based prospective cross-sectional study.

Source of data and sample size

59 cases of primary breast carcinoma MRM specimens submitted to the Department of Pathology, Silchar Medical College and Hospital, Silchar, for histopathological examination (Figure 1). Immunohistochemistry with CyclinD1 and ER antibody was done on these specimens as per IHC protocol.

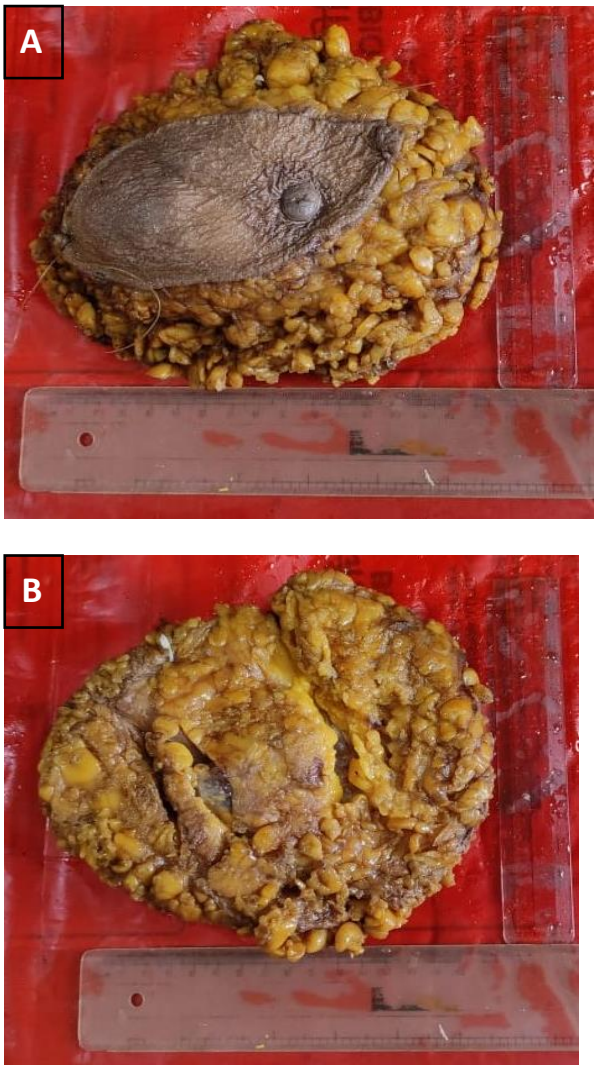


Figure 1. Gross pictures an MRM specimen (A is anterior view; B is posterior view).

Inclusion criteria

In the study, patients with invasive duct carcinoma, no special type (IDC, NST) as histopathological diagnoses were included.

Exclusion criteria

- All metastatic carcinoma of breasts.

- Male breast carcinomas

Parameters studies

I. Detailed clinical history is taken and all routine investigations are done after obtaining consent from the patients.

II. Hospital records of the patients.

III. Microscopic examination of the tissues.

IV. Immunohistochemistry on paraffin embedded tissue of histopathologically diagnosed cases.

The current study was conducted prospectively at a hospital in Silchar, India, in the Department of Pathology during a year, from 2021 to 2022. 59 biopsy/resection specimens for primary breast carcinoma were submitted in total. All regular investigations are carried out after obtaining the patients' agreement and a thorough clinical history is gathered. These specimens were first stained with H&E before being subjected to immunohistochemistry using CyclinD1 and ER antibodies by the IHC methodology.

Preparation of slides: Paraffin sections were cut and mounted on saline coated slides. The slides were heated at 65℃ to remove the paraffin and then immerse in xylene. After rehydration of the tissues, the slides were cleaned with distilled water. Subsequently, the slides were washed with Tris buffer and submerged in a 3% peroxide solution for three minutes to remove endogenous peroxidase activity.

Antigen detection and antigen retrieval: Heat retrieval was performed using a decloaking chamber with citrate buffer at 95℃ for 40 minutes. The slides were then transferred to Tris-Saline buffer to cool to room temperature. To prevent non-specific immunostaining , the tissue sections were treated with 1% mouse serum. Primary antibodies, including Rabbit monoclonal antibody QR022 for CyclinD1 and Rabbit monoclonal antibody QR013 for ER were applied to the sections approximately one hour before removal.

Secondary detection of the primary antibody: After 10 minutes of incubation with biotinylated mouse anti-species antibody, sections were washed in Tris buffer. The slides were then treated with a solution of chromogen 3,3'- diaminobenzidine (DAB) at a concentration of 1mg/mL in Tris buffer containing 0.016% fresh H2O2. Tap water was used to clean the DAB from the slides.

Counterstaining: Slides were immersed in a solution of hematoxylin diluted 1:1 with distilled water for

counterstaining. After counterstaining, the slides were cleaned in distilled water and dehydrated by dipping them in ethanol. Then a coverslip was used to view and report after cleaning in xylene (Figure 2).

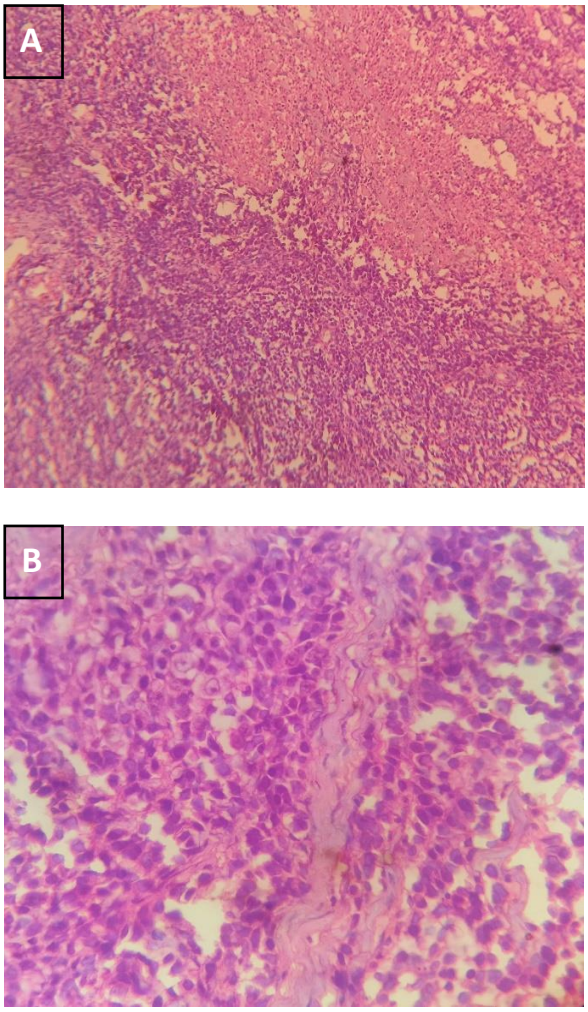


Figure 2. H&E pictures of IDC, NST (A: 10X and B: 40X).

Reporting of CyclinD1 immuno-histochemical study

A semi-quantitative scoring is used by the Allred score method for the nuclear staining (11) as

- 0: negative (no staining of any nuclei even at high magnification)
- 1: weak (only visible at high magnification)
- 2:moderate (readily visible at low magnification)
- 3: strong (strikingly positive even at low power magnification).

Additionally noted was the percentage of tumor nuclei that stained positively as:

0- none, **1**- <1/100, **2**- 1/100 to 1/10, **3**- 1/10 to 1/3, **4**- 1/3 to 2/3 and

5- >2/3.

After that, the intensity scores and proportion were combined to get a final score that varied from 0 to 8 (11).

Tumors were then categorized as:

- Negative/weak expression (total scores 0–2)
- Intermediate expression (total scores 3–5)
- Strong expression (total scores 6–8)

In this study, Intermediate and Strong positives were considered together as positive.

Reporting of ER immunohistochemical study

Strong brown to black nuclear staining was considered when assessing immune positivity for ER. Positive nuclei were expressed as the percentage of total nuclei counted.

Criteria for evaluating ER (12)

- **Negative for ER**: If, 1% or 0% of tumor cell nuclei are immunoreactive.
- **ER Low Positive**: If 1%-10% of tumor cell nuclei are immunoreactive.
- **Positive for ER**: 1%-100% of tumor nuclei are immunoreactive.

Statistical analysis

IBM SPSS software version 21.0 was used for data analysis. Qualitative data was presented as frequency and percentage, while quantitative data was presented as mean (±SD). The chi-square test was used to identify significant associations. A p-value of <0.05 was regarded as statistically significant.

Results

In our study, various clinicopathological parameters are analyzed and are presented as under.

The mean age of the patients having breast carcinoma was 52.12 ± 12.47 years and the majority of the patients belonged to 41 to 50 years of age (32.2%). This was followed by 28.8% and 18.6% cases belonging to the age range of 51 to 60 years and ≤40 years of age respectively (Table 1).

Table 1. Distribution according to age.

Age (in years)	Frequency (n = 59)	Percentage (%)
≤40	11	18.6
41 – 50	19	32.2
51 – 60	17	28.8
61 – 70	07	11.9
>70	05	8.5
Mean	52.12 ± 12.47	

In the present study, right-side predominance was observed for breast carcinoma. 52.5% of patients had carcinoma breast on the right breast while 47.5 % were over the left breast. (Table 2).

Table 2. Distribution according to laterality of breast carcinoma.

Laterality	Frequency (n = 59)	Percentage (%)
Left	28	47.5
Right	31	52.5

In our study, 33.9% of patients were in a pre-menopausal state and a majority of the cases 66.1% were in the post-menopausal phase. We considered menopause where no menstruation was reported over the last 12 months. (Table 3).

Table 3. Distribution according to menopausal status.

Menopausal status	Frequency (n=59)	Percentage (%)
Pre-menopausal	20	33.9
Post –menopausal	39	66.1

Most commonly affected (40.7%) cases of breast carcinoma patients had tumour of size 2-5cm. This was followed by ≤2cm tumour size in 30.5% and >5cm in 28.8% cases respectively (Table 4).

Table 4. Distribution according to tumour size.

Size of tumour	Frequency (n = 59)	Percentage (%)
≤2cm	18	30.5
2-5cm	24	40.7
>5cm	17	28.8

In our study, lymph node metastasis was observed in 47.5% of cases whereas, in 52.5% of cases, no lymph node metastasis was documented (Table 5) (Figure 3).

Table 5. Distribution according to lymph node metastasis of Breast carcinoma.

Metastasis	Frequency (n = 59)	Percentage (%)
Present	28	47.5
Absent	31	52.5

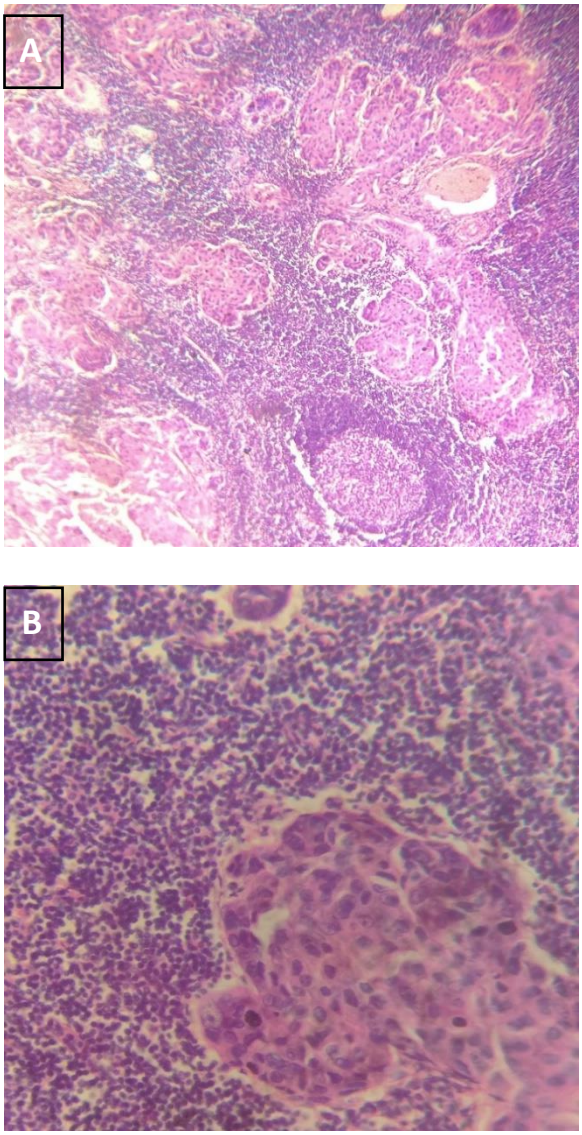


Figure 3. H&E picture of metastatic lymph node (A: 10X and B: 40X).

The Nottingham (Elston-Ellis) modification of the Scarff-Bloom-Richardson grading system also called as the Nottingham Grading System is applied for the above grading. The majority of cases 61% were found to be in grade II, this was followed by 30.5% and 8.5% in grade III and I respectively (Table 6).

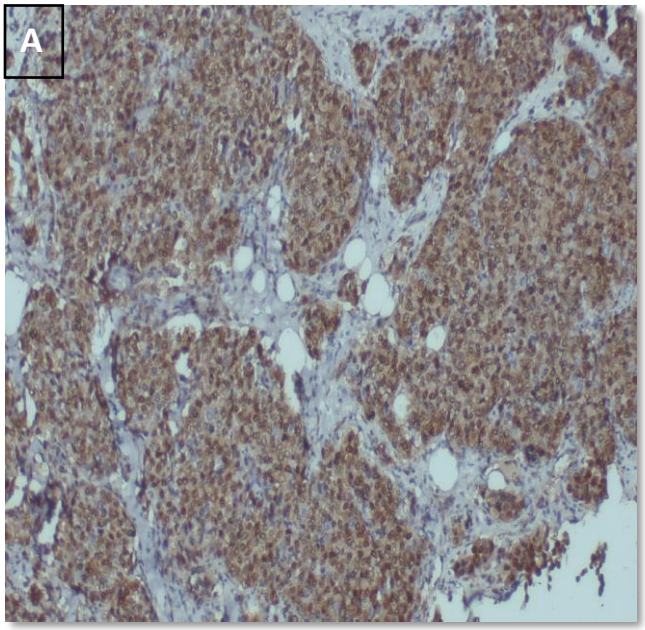
Table 6. Distribution according to the histological grades of the tumours.

Tumour grade	Frequency (n=59)	(%)
I	5	8.5
II	36	61
III	18	30.5

Tumour cells with >10% nuclear staining were regarded as positive and <10% or weak staining as negative. In this study, we found 36 out of 59 cases (61%) showed CyclinD1 positive expression whereas 23 cases (39%) cases had negative CyclinD1 expression (Table 7) (Figure 4).

Table 7. Distribution according to the expression of CyclinD1 in breast carcinoma.

CyclinD1 expression	Frequency (n = 59)	Percentage (%)
Positive	36	61
Negative	23	39



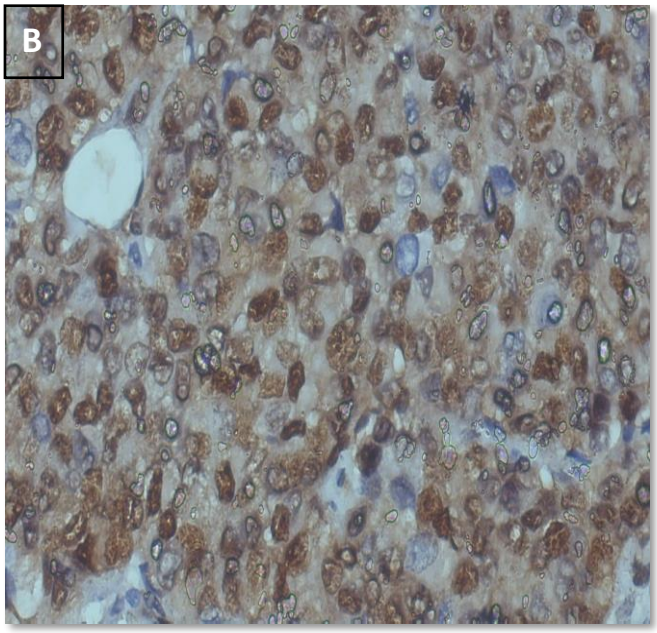


Figure 4. CyclinD1 positive in a case of IDC, NST (A: 10X, B: 40X).

For calculating the statistical significance, we grouped 59 cases into 2 categories based on their age as ≤40 years and >40 years age. The majority of patients, 48 out of 59(81.4%) are over 40 years of age and 11 (18.6%) are ≤40 years of age. 94.4% of patients >40 years show positive CyclinD1 expression and 60.9% of cases are negative for CyclinD1. However, 5.6% of cases of ≤40 years of age show positive CyclinD1 expression. The test of significance (chi-square test) showed a statistically significant association between age and CyclinD1 expression in the present study ($\chi 2$ = 8.334, P-value=0.0039) (Table 8).

Table 8. Association between age and CyclinD1 expression.

Age (in years)	Total cases (n=59)	CyclinD1 positive (n=36)	CyclinD1 negative (n=23)	P-value
≤40	11 (18.6%)	2(5.6%)	9(39.1%)	
41-50	19 (32.3%)	14 (38.9%)	5 (21.7%)	0.0039
51-60	17 (28.8%)	10(27.8%)	7 (30.4%)	
61-70	07 (11.9%)	06(16.6%)	01(4.4%)	
>70	05 (8.5%)	04(11.1%)	01 (4.4%)	

For calculating the p-value, we grouped the tumour size into 2 categories: ≤2 (18 cases) and >2cm (41 cases). CyclinD1 expression was seen in 47.2% of tumours with ≤2cm tumour size and 52.8%tumours with size >2cm. The difference was statistically significant ($\chi 2$ = 10.230, P-value=0.014). Also, the majority (65.2%) of CyclinD1 negative tumours have a size >5cm, followed by 2-5cm and ≤2cm with 30.4% and 4.4% respectively. This shows that with an increase in tumour size there is an increase in Cyclin-D1 negativity (Table 9).

Table 9. Association between tumour size and CyclinD1 expression.

Tumour size (in cm)	Total cases (n=59)	CyclinD1 positive (n= 36)	CyclinD1 negative (n=23)	P-value
≤2	18 (30.5%)	17 (47.2%)	01 (4.4%)	
2-5	24 (40.7%)	17 (47.2%)	07 (30.4%)	0.014
>5	17 (28.8%)	02 (5.6%)	15 (65.2%)	

In this study, from 36 overexpressed Cyclin-D1 cases, the majority of cases 80% are in grade I. This is followed by grade II and grade III with 69.4% and 38.9% cases respectively. For calculating the statistical significance (p-value) of this correlation, we grouped grade I and II as intermediate grade and grade III alone as high grade. This correlation was found to be statistically significant (p-value=0.0435). This implies Cyclin-D1 nuclear positivity is associated with lower tumour histological grade (Table 10).

In this study, from 40 ER-positive cases, the majority of cases 80% are in grade I. This is followed by grade II and grade III with 69.4% and 61.1% cases. For calculating the statistical significance (p-value) of this

correlation, we grouped grade I and II together as intermediate grade and grade III alone as high grade. This correlation was found to be statistically insignificant (p-value>0.05) (Table 10).

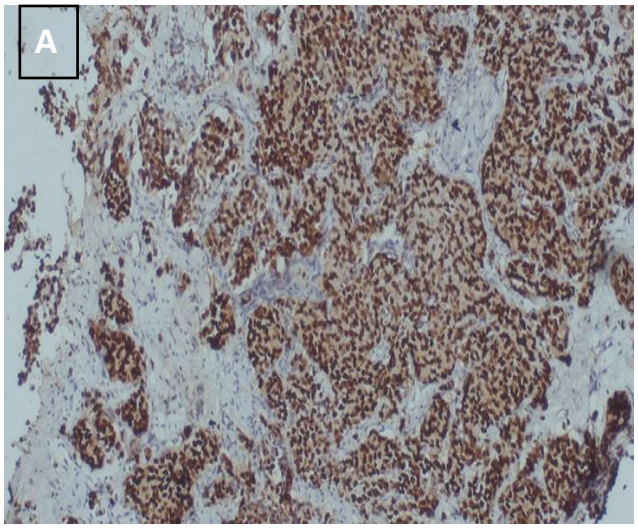
Table 10. Correlation between Cyclin-D1, ER expression and histological grade of tumors.

Grade	n	Cyclin-D1 positive	Cyclin-D1 negative	P-value	ER positive	ER negative	P-value
I	5	4(80%)	1 (20%)		4 (80%)	1(20%)	
II	36	25(69.4%)	11 (30.6%)	0.0435	25 (69.4%)	11(30.6%)	>0.05
III	18	7(38.9%)	11 (61.1%)		11 (61.1%)	7(38.9%)	

In the present study, the majority of breast carcinoma patients 40 out of 59 (67.8%) had positive ER expression whereas ER-negative expression was observed in 32.2% of cases (Table 11) (Figure 5).

Table 11. Distribution according to the expression of ER in breast carcinoma.

ER expression	Frequency (n=59)	Percentage (%)
Positive	40	67.8
Negative	19	32.2



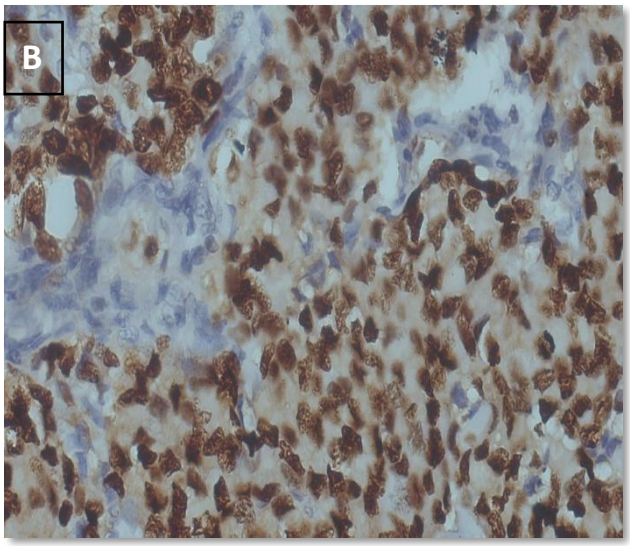


Figure 5. ER positive in a case of IDC, NST (A: 10X, B: 40x).

In this study, 40 ER-positive cases were found and expressed CyclinD1 in 100% of cases and 17.4% showed no expression for CyclinD1. Of 19 ER-negative cases, 82.6% were negative for CyclinD1. The association between CyclinD1 and ER expression was observed to be statistically significant ($\chi 2$ =40.163, P value= 0.001) (Table 12).

Table 12. Association between CyclinD1 and ER expression in breast carcinoma.

ER-expression	Frequency (n=59)	CyclinD1 positive (n=36)	CyclinD1 negative (n=23)	P-value
Positive	40(67.8%)	36 (100%)	04 (17.4%)	0.001
Negative	19(32.2%)	0(0%)	19 (82.6%)	

Among 20 pre-menopausal women, 15% expressed ER positivity while 73.7% of cases were ER-negative. Of 39 post-menopausal women, 85% showed ER positivity and 26.3% were ER negative. The difference was statistically significant (P-value< 0.05). This implies that there is increased ER expression in post-menopausal breast carcinoma patients (Table 13).

Table 13. Association between menopausal status and ER expression.

Menopausal status	Frequency (n=59)	ER-positive (n=40)	ER-negative (n=19)	P-value
Pre-menopausal	20(33.9%)	6(15%)	14(73.7%)	< 0.05
Post - menopausal	39(66.1%)	34(85%)	5(26.3%)	

Out of 11 cases of ≤40 years of age, ER expression was observed in 05% of cases. Of 48 cases in the>40 years age group, 95% showed positive ER expression. The difference was found to be statistically significant ($\chi 2$ = 4.477, P-value=0.0004). This implies that ER-positive expressions were more common in >40 years old patients with breast carcinoma (Table 14).

Table 14. Association between age and ER expression.

Age (in years)	Frequency (n=59)	ER-positive (n=40)	ER-negative (n=19)	P-value=
≤40	11 (18.6%)	2 (05%)	9 (47.4%)	0.0004
>40	48 (81.4%)	38 (95%)	10 (52.6%)	

Discussion

The present study includes 59 patients presenting to our institute with a breast lump that underwent biopsy and were diagnosed as intra-ductal carcinoma on histopathological examination from August 2021 to July 2022. The incidence was assessed with age, menopausal state, and laterality by carefully examining the patient profiles. A thorough investigation of Cyclin-D1 and ER expression was done using immunohistochemistry.

Among the 59 cases of breast carcinomas, the maximum cases (32.2%) were in the age group 41-50 years followed by 28.8%, and 18.6% cases from the age group 51-60 years and <40 years respectively. This is followed by the age group 61-70 years and >70 years respectively with each 11.9% and 8.5%. This is in concordance with Lengare PV et al. (2020) (13) where the maximum number of patients 38% lie in the age group 41-50 years.

Most of the patients in our study were in the 5th decade with a mean age of 52.12±12.47 years. This is in concordance with the study done by Servet K. et al (2019)(14), Lengare PV et al. (2020) (13) and Mohammadizadeh F et al. (2013) (15).

Among the 59 cases of breast carcinoma, 52.5% cases were reported from the right breast and 47.5% cases from the left breast.

In this study, majority of the cases (66.1%) were post-menopausal which is similar to studies conducted by Mazor M et al. (2018) (16), Singh R et al (2014) (17) and Roy et al (2010) (18) with 62.6%, 64% and 64% respectively.

This study showed that majority (40.7%) of tumour size were more than 2-5cm followed by ≤2cm tumour with 30.5% and >5cm tumour with 28.8%. This study was found in concordance with Servet K et al (2019)

(19), Ortiz AB et al (2017) (20), Bilalović N et al (2005) (21), Lee A et al (2007) (22) and Li Z et al(2016) (23).

As per this study, 47.5% of cases showed lymph node metastasis in breast carcinoma. Other significant studies where nodal metastasis were noted in breast carcinoma cases were Lengare PV et al (2020) (13) with 68% cases, Roy et al (2010) (18) with 51.5% cases, Ortiz AB et al (2017) (20) with 48% cases, Lee A et al (2007) (22) with 48.9% cases, Azizun-Nisa et al (2008) (24) with 71.3% cases, Peurala E et al (2013)(25) with 41.2% cases, Boström P et al(2009)(26) with 52.8% cases, Khabaz MN. (2014) (27) with 53.5% cases.

In the present study, Cyclin-D1 immune expression was positive in 36 out of 59 cases (61%) and negative in 23 cases (39%). Similar studies showing Cyclin-D1positivity are Mohammadizadeh F et al (2013) (15) with 78.6%, Reis-Filho JS et al (2006) (28) with 67.4%, Ortiz AB et al (2017)(20) with 52%, Lengare PV et al(2020) (13) with 64% , Peurala E et al. (2013) (25) with 60%, Siraj AK et al. (2021) (29) with 59.4%, Roy P. G et al. (2010) (18) with 63.4% and Lee A et al (2007) (22) with 63.9%.

In our study, it is found that with an increase in the age of patients, there is also an increase in the expression of CyclinD1 in breast carcinoma which is in concordance with Li Z et al (2016) (23) that showed 86.4% of CyclinD1 positive cases were ≥35 years and 13.6% were <35 years and Siraj AK et al (2021) (29) with 61.2% >50 years and 58.6% ≤50 years breast cancer patient expressing CyclinD1.

In the present study, out of 59 cases of breast carcinoma, ER was positive in 40 cases (67.8%) and negative in 19 cases (32.2%). This is in concordance with Roy et al (2010) (18) with ER positivity of 76.8%, Siraj AK et al (2021)(29) with 65.6%, Peurala E et al (2013)(25) with 76.5%, Mostafa M et al (2010) (30)with 69%, Singh R et al (2014) (17) with 44.6%, Bilalović N et al (2005) (21) with 79%, Lee A et al (2007)(22) with 64%, Mohammadizadeh F et al (2013)(15) with 60.7% and Lengare PV et al(2020) (13) with 56%.

In this study, 90% cases show ER-positivity in Cyclin-D1-positive breast carcinoma. Similar findings were found in studies conducted by Lee A et al (2007) (22) with 77.8% of Cyclin-D1 positive cases showing ER-positivity, Elsheikh, et al (2008) (31) with 54.6%, Lengare PV et al(2020) (13) with 54%, Li Z et al (2016) (23) with 81.2% , Roy et al (2010) (18) with 69.7%, Peurala E et al (2013) (25) with 96.6%, Reis-Filho JS et al (2006) (28) with 76.3% and Siraj AK et al. (2021) (29) with 72.6%.

85% of post-menopausal women show ER-positivity in breast carcinoma in our studies. This is in concordance with Singh R et al (2014) (17) with 48.4%, Md. Oliul Islam et al (2022) (32) with 57.1%.

In our study, ≤40 years women showed 05% ER positivity and >40 years patients showed 95% ER positivity which is statistically significant. This implies that there is a trend of higher ER positivity in older breast carcinoma patients (<40 years) than in younger (≤40 years).

This study is in concordance with Singh R et al (2014) (17) and Mostafa M et al (2010) (30) where among ER-positive cases, the maximum breast carcinoma cases were of the older age group (>50 years) with 50.9% and 69.1% respectively. Also, Aysha S. AlZaman et al (2016) (33) showed similar findings with ER-positive breast carcinoma having 72.6% of >40 years patients.

Conclusion

Regardless of regional variances, carcinoma of breast is the most frequent cancer in women. Among other things, the incidence of intra-ductal breast cancer is still very high.

Recent years have seen an increase in the early diagnosis of breast carcinoma due to increased public awareness of breast cancer and breast self-examination. This should be backed up by an immune-histochemical analysis of the numerous hormone receptors to pinpoint the cases that may respond well to hormonal therapy, extending the patients' disease-free survival.

In the current study, we found that the majority of breast cancer cases were seen in the fifth decade, then the sixth decade, and that the majority of patients were post-menopausal, with the right breast predominating.

In this study, the majority of cases were in grade II, followed by grade III and grade I.

The majority of the patients were positive for immune-staining for CyclinD1 and ER and were found to be associated with low histological grades.

This study demonstrated a trend towards increased CyclinD1 and ER-positive with aging.

We discovered that Cyclin-D1 positivity decreases and Cyclin-D1 negativity increases as the tumor growth increases.

The majority of post-menopausal patients had ER-positive, highlighting the fact that radiation and chemotherapy with anti-estrogens (such as Tamoxifen) may be beneficial in such instances.

Since CyclinD1 and ER-positive cases had better treatment outcomes than those negative tumors, they are now indicated as clinical prognostic markers for IDS, NST patients.

Limitations

The small number of cases due to SARS-COV-2 infections and the absence of post-operative information for the cases we analyzed.

Conflict of interests

The authors declare that they have no competing interests.

Authors contributions

All the authors have contributed equally and read and approved the final draft of the manuscript.

Funding

There is no funding.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49.

2. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, Vignat J, Gralow JR, Cardoso F, Siesling S, Soerjomataram I. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast. 2022 Dec;66:15-23.

3. Martelli G, Pilotti S, De Yoldi C, Viganotti G, Fariselli G, Lepera G, et al. Diagnostic efficacy of physical examination, Mammography, fine needle aspiration cytology (triple test) in solid lumps. An analysis of 1708 consecutive cases. Tumori. 1990;76(5):476–9.

4. Johnson RH. Incidence of Breast Cancer With Distant Involvement Among Women in the United States, 1976-2009 (vol 309, pg 800, 2013). JAMA-JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION. 2013 Mar 27;309(12):1229-.

5. Fredholm H, Eaker S, Frisell J, Holmberg L, Fredriksson I, Lindman H. Breast cancer in young women: poor survival despite intensive treatment. PloS one. 2009 Nov 11;4(11):e7695.

6. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast cancer before age 40 years. Semin Oncol. 2009 Jun;36(3):237-49.

7. Stricker TP, Kumar V, Abbas AK, Fausto N, Ster JC. Robbins and Cotran pathologic basis of disease.

8. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. Breast cancer research and treatment. 1992 Oct;22(3):207-19.

9. Fouad YA, Aanei C. Revisiting the hallmarks of cancer. American journal of cancer research. 2017;7(5):1016.

10. Harris JR, Lippman ME, Osborne CK, Morrow M. Diseases of the Breast. Lippincott Williams & Wilkins; 2012 Mar 28.

11. Harvey JM, Clark GM, Osborne CK, Allred DC. 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. 1999 May 1;17(5):1474-81.

12. Allison KH, Hammond ME, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, Perou CM. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. Journal of Clinical Oncology. 2020 Apr 20;38(12):1346-66.

13. Lengare PV, Khandeparkar SG, Joshi AR, Gogate BP, Solanke SG, Gore SH. Immunohistochemical expression of cyclin D1 in invasive breast carcinoma and its correlation with clinicopathological parameters. Indian Journal of Pathology and Microbiology. 2020 Jul 1;63(3):376.

14. Kocaöz S, Korukluoğlu B, Parlak Ö, Doğan HT, Erdoğan F. Comparison of clinicopathological features and treatments between pre-and postmenopausal female breast cancer patients–a retrospective study. Menopause Review/Przegląd Menopauzalny. 2019 Jun 14;18(2):68-73.

15. Mohammadizadeh F, Hani M, Ranaee M, Bagheri M. Role of cyclin D1 in breast carcinoma. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2013 Dec;18(12):1021.

16. Mazor M, Cataldo JK, Lee K, Dhruva A, Paul SM, Smoot BJ, Dunn LB, Levine JD, Mastick J, Conley YP, Miaskowksi C. Influence of Menopausal Status on the Symptom Experience of Women Prior to Breast Cancer Surgery. Cancer nursing. 2018 Jul;41(4):265.

17. Singh R, Gupta S, Pawar SB, Pawar RS, Gandham SV, Prabhudesai S. Evaluation of ER, PR and HER-2 receptor expression in breast cancer patients presenting to a semi urban cancer centre in Western India. Journal of cancer research and therapeutics. 2014 Jan 1;10(1):26.

18. Roy PG, Pratt N, Purdie CA, Baker L, Ashfield A, Quinlan P, Thompson AM. High CCND1 amplification identifies a group of poor prognosis women with estrogen receptor positive breast cancer. International journal of cancer. 2010 Jul 15;127(2):355-60.

19. Kreike B, van Kouwenhove M, Horlings H, Weigelt B, Peterse H, Bartelink H, van de Vijver MJ. Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Research. 2007 Oct;9(5):1-4.

20. Ortiz AB, Garcia D, Vicente Y, Palka M, Bellas C, Martin P. Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PloS one. 2017 Nov 15;12(11):e0188068.

21. Bilalović N, Vranić S, Bašić H, Tatarević A, Selak I. Immunohistochemical evaluation of cyclin D1 in breast cancer. Croatian medical journal. 2005 Jun 1;46(3).

22. Lee A, Park WC, Yim HW, Lee MA, Park G, Lee KY. Expression of c-erbB2, cyclin D1 and estrogen receptor and their clinical implications in the invasive ductal carcinoma of the breast. Japanese journal of clinical oncology. 2007 Sep 1;37(9):708-14.

23. Li Z, Cui J, Yu Q, Wu X, Pan A, Li L. Evaluation of CCND1 amplification and CyclinD1 expression: diffuse and strong staining of CyclinD1 could have same predictive roles as CCND1 amplification in ER positive breast cancers. American journal of translational research. 2016;8(1):142.

24. Azizun-Nisa BY, Raza F, Kayani N. Comparison of ER, PR and HER-2/neu (C-erb B 2) reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer. Asian Pac J Cancer Prev. 2008 Oct;9(4):553-6.

25. Peurala E, Koivunen P, Haapasaari KM, Bloigu R, Jukkola-Vuorinen A. The prognostic significance and value of cyclin D1, CDK4 and p16 in human breast cancer. Breast Cancer Research. 2013 Feb;15(1):1-0.

26. Boström P, Söderström M, Palokangas T, Vahlberg T, Collan Y, Carpen O, Hirsimäki P. Analysis of cyclins A, B1, D1 and E in breast cancer in relation to tumour grade and other

prognostic factors. BMC research notes. 2009 Dec;2(1):1-8.

27. Khabaz MN. Immunohistochemistry subtypes (ER/PR/HER) of breast cancer: where do we stand in the West of Saudi Arabia?. Asian Pacific Journal of Cancer Prevention. 2014;15(19):8395-400.

28. Reis-Filho JS, Savage K, Lambros MB, James M, Steele D, Jones RL, Dowsett M. Cyclin D1 protein overexpression and CCND1 amplification in breast carcinomas: an immunohistochemical and chromogenic in situ hybridisation analysis. Modern pathology. 2006 Jul;19(7):999-1009.

29. Siraj AK, Parvathareddy SK, Annaiyappanaidu P, Ahmed SO, Siraj N, Tulbah A, Al-Dayel F, Ajarim D, Al-Kuraya KS. High Expression of Cyclin D1 is an Independent Marker for Favorable Prognosis in Middle Eastern Breast Cancer. OncoTargets and therapy. 2021;14:3309.

30. Mostafa MG, Larsen MT, Love RR. Estrogen receptor, progesterone receptor, and Her-2/neu oncogene expression in breast cancers among Bangladeshi women. Journal of Bangladesh College of Physicians & Surgeons. 2010;28(3):157.

31. Elsheikh S, Green AR, Aleskandarany MA, Grainge M, Paish CE, Lambros MB, Reis-Filho JS, Ellis IO. CCND1 amplification and cyclin D1 expression in breast cancer and their relation with proteomic subgroups and patient outcome. Breast cancer research and treatment. 2008 May;109(2):325-35.

32. Islam MO, Sultana F, Khan RM, Islam AZ, Rana MS, Nasrullah SS, Hossain MF, Ahmed SU, Mubin S. Relation of Receptor Status and Tumor Grade with Menopausal Status in Patients of Carcinoma Breast. SAS J Surg. 2022 Mar;3:134-42.

33. AlZaman AS, Mughal SA, AlZaman YS, AlZaman ES. Correlation between hormone receptor status and age, and its prognostic implications in breast cancer patients in Bahrain. Saudi medical journal. 2016 Jan;37(1):37.