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Original Research Article

Extensive intraductal component positive carcinoma of breast: two year study with special reference to ER/PR/HER2NEU/Ki67 in a tertiary care centre of Barak Valley

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ABSTRACT

Background: Extensive intraductal component positive carcinoma (EICPC) of breast is defined by Schnitt et al as- A. 25% or more of Ductal carcinoma in situ (DCIS) is present along the invasive lesion and DCIS is also present outside the area of invasive carcinoma. B. EICPC also include carcinomas in which DCIS is associated with a "small" (approximately 10 mm or less) invasive carcinoma or carcinomas. In Extensive Intraductal Carcinoma (EIDC) most of the cases were associated with recurrence when surgical margin status is not evaluated or focally involved. Our objective was to study the prevalence of EIDC and expression of estrogen receptor (ER)/progesterone receptor (PR)/human epidermal growth factor (HER2NEU)/Ki67(antigen identified by monoclonal antibody KI67) in those cases.
Methods: It was a retrospective cross sectional study conducted over a period of 2017 to August 2019.All the histologically confirmed cases of EIDC was retrieved from the institute.
Results: Out of 65 cases of invasive carcinoma 17 (26.1%) cases were positive for EICPC. Age of patients ranged from 27 to 73years with mean age of 43 years and 5 patients (29.4%) were postmenopausal. Most of the cases i.e. 6(35.2%) had a ER+/PR+/HER2NEU- status with most of the cases having high 6(47%)Ki-67 index. According to the BLOOM RICHARDSON GRADING 14 cases were grade II (82.3%) and 3 cases were grade I (17.7%) and in pT and pN staging majority were stage pT1 - 7 (41.1%). Most of the cases were mastectomy cases 11 (64.4%) with a base free status except in one lumpectomy case where margin was involved.
Conclusions: In this study majority of the cases were ER+//PR+/HER2NEU- with most of the cases having high Ki67 index. Evaluation of EIDC, along with the negative margin status is important to prevent recurrence.

Keywords: Ductal carcinoma in situ, Extensive intraductal carcinoma, Invasive carcinoma

INTRODUCTION

Carcinoma of the breast is the second most common non-skin malignancy in woman and is second only to lung cancer as a cause of cancer deaths. Almost all (>95%) of breast malignancies are adenocarcinomas that arise in the duct/lobular system as carcinoma in situ. Ductal Carcinoma in Situ (DCIS) is a malignant clonal proliferation of epithelial cells limited to ducts and lobules by the basement membrane.[1] For several decades it has been accepted that DCIS constitutes a non-obligate precursor of invasive ductal carcinoma.

It has been postulated that in patients with infiltrating ductal breast cancer treated with conservative surgery and radiotherapy, the presence of an extensive intraductal component (EIC) in the initial excision specimen is highly associated with subsequent recurrence.[2]

EIC positive carcinomas represent almost 5% of breast cancer, but few data exist concerning their characteristics and prognostic behaviour.[3] Extensive intraductal component positive carinoma (EICPC) is defined as Schnitt et al as.[4,5]

- ≥25% of the area within the invasive carcinoma is ductal carcinoma in situ (DCIS) and
- DCIS is also present outside the area of invasive carcinoma.[3]

- EICPC also include carcinomas in which DCIS is associated with a "small" (approximately 10 mm or less) invasive carcinoma or carcinomas.[3]

In the current study we studied about the prevelance of various parametres like age, grade, stage in EIDC positive cases, along with special reference to ER/PR/HER2NEU/Ki67 status in those cases.

The presence of the hormone receptors ER, PR in a patient's breast cancer is an example of a weak prognostic but strong predictive biomarker.

If a patient's tumor expresses ER and/or PR, we can predict that this patient will positively benefit from endocrine therapy such as tamoxifen.

The overexpression of the oncogene HER2NEU in a patient's breast cancer is an example of both a prognostic and predictive biomarker. HER2NEU expression is associated with poor prognosis (high risk of recurrence [ROR]); however, it also predicts that a patient will more likely benefit from anthracycline and taxane-based chemotherapies and therapies that target HER2NEU (trastuzumab), but not to endocrine-based therapies.[6]

METHODS

It was a retrospective cross sectional study conducted over a period of 2017 to August 2019.

All the histologically confirmed cases of EIDC was retrieved from the institute. All the other types of invasive carcinomas with or without in situ component which are not fitting to the definition of EIC positive cancer were excluded.

Out of 65 cases of invasive cariconoma, 17 cases were found to be EIDC.

Mean age group was found out for those cases in which there was highest expression of EIDC. These cases were then evaluated for ER/PR/Her2neu/Ki67 status.

ER and PR are hormone receptors found on breast cells that pick up hormone signals resulting in cell growth.

Allred scoring system was used to access estrogen and progesterone positive receptor.

In Allred system of scoring, score 0-5 is given to the cells depending on the proportion of cells which are stained (proportion score [PS]) and score 0-3 is given depending on the intensity of staining (intensity score [IS]). By adding the PS and IS, we can calculate the final Allred score (PS + IS = AS).

Total score of 0-2 considered as negative,whereas between 3-8 considered as positive. HER2/neu is a cell membrane receptor which helps in cell division,growth and repair. Table 1 the scoring of human epidermal growth factor receptor-2/neu was accessed depending on the intensity of staining of cells.

Table 1: Scoring of HER2NEU expression.

Staining pattern	Score	HER2NEU overexpression
No staining is observed/membrane staining is observed in <10% of the tumour cells	0+	Negative
A faintly perceptible membrane staining is detected in >10% of the tumour cells. The cells are only stained in part of their membrane.	1+	Negative
A weak to moderate complete membrane staining is observed in >10% of cells	2+	Weakly positive/equivocal
A strong complete membrane staining is observed in >30% of the tumour cells.	3+	Strongly positive.

Ki-67 protein in humans is encoded by MK167 gene and is a cellular marker for proliferation. This is a nuclear protein and is expressed in proliferating cells but is not detected in resting cells and is one of the most reliable indicators of the proliferative status of cancer cells. Ki-67 expression was detected by staining with MIB-1 antibody.

In breast cancer Ki67 is evaluated as follows:

Low- <10%
Borderline - 10-20%
High - >20%

Grading of these cases were done by Modified Bloomrichardson grading where it includes 3 criteria i.e. - tubules formation, nuclear pleomorphism and mitotic

count which grades the tumour in 3 grades (GradeI-3-5 points, GradeII-6-7 points and GradeIII-8-9 points).

These cases were also evaluated for pT staging by taking into account the tumour size, where there is no gross tumour at the margin of resection.

Finally the cases were observed for the type of surgery they underwent and the margin status was evaluated.

RESULTS

Out of 65 cases of invasive breast carcinoma from August 2017 to August 2019, 17 cases were positive for EIDC (Figure1) that were included in the study.

In the figure 1 showing extensive intraductal component, with more than 25% of the invasive carcinoma is ductal carcinoma in situ, fitting into the first definition of EIDC. EIDC positive primary tumours commonly have a large amount of cancer (predominantly intraductal carcinoma) beyond the edge of the primary tumour.

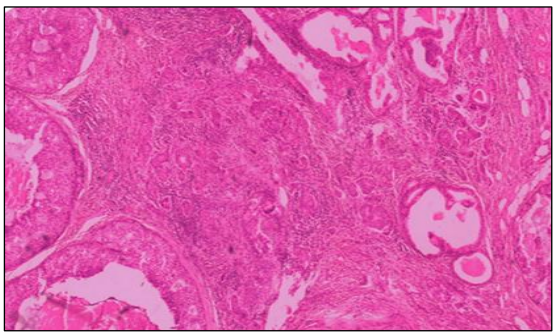

Figure 1: HnE features of invasive breast carcinoma, with 25% of areas with features of DCIS.

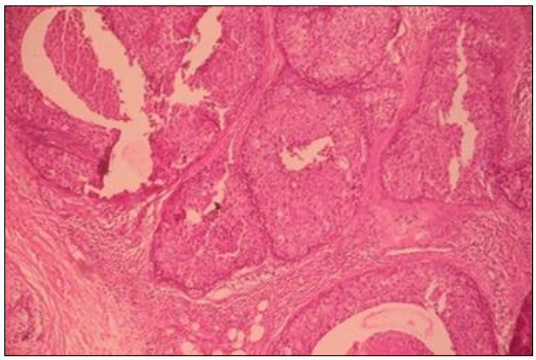

Figure 2: EIDC with microinvasion.

EIDC also include carcinoma in which the DCIS component shows small approximately 10mm or less invasive carcinoma or carcinomas as already discussed in the second definition of EIDC. Figure 2 showing HnE stained slide, with microinvasion and the rest showing DCIS component.

The age ranged from 20-80 years. Most of the cases were in the range of 40-60 years, with a mean age group of 43 years and 5 patients were in the post menopausal age group 6 cases were found in the age group of 20-40 years and only 2 cases were found in the range of 60-80 years (Figure 3). So most of the cases were found in the perimenopausal group.

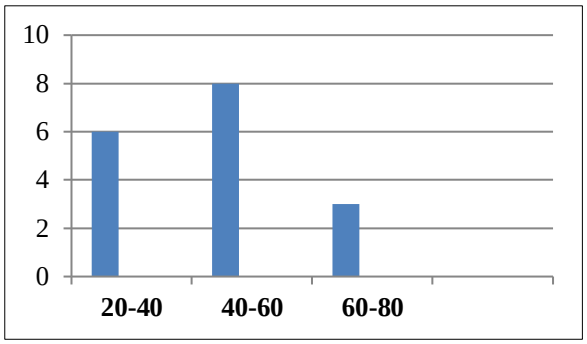

Figure 3: Age wise distribution of cases.

Tumours were classified according to the BLOOM and RICHARDSON grading system. The BRG system includes number of tubule formation, nuclear size and mitotic count. Figure 4 showing the distribution of cases according to the grade which include the above three features. They are described as follows:

Tubule formation

- SCORE 1: >75% of the tumour shows tubules.
- SCORE 2: 10-75% of the tumour has tubules.
- SCORE 3: <10% of the tumour has tubules.

Nuclear size

- SCORE 1: small regular nuclei,similar to normal ductal nuclei
- SCORE 2: intermediate size;1.5-2 times the size of normal ducat nuclei
- SCORE 3: high grade nuclei;>twice the size of normal ductal nuclei.

Mitotic count

- SCORE 1: 0-7 mitosis/10HPF
- SCORE 2: 8-14/10HPF
- SCORE 3: >15/HPF

Nottingham combined histologic score

- SCORE 3-5: GRADE I
- SCORE 6-7: GRADE II
- SCORE 8-9: GRADE III

In this study, 14 cases were in grade II (82.3%) and 3 cases were in grade I (17.7%). No cases were found in grade III (Figure 4).

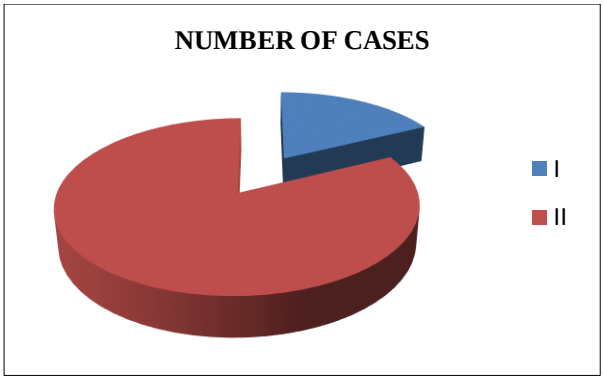

Figure 4: BRG grading frequency.

The tumour size was recorded as the greatest diameter microscopically. Regarding pathological tumour size out of 17 cases of EIDC most of the cases were in pT1 i.e. 7 cases (41.1%) followed by pT2 (6cases- 29.4%) and least common is pT3 (2case- 5.8%). 2 cases were seen to have microinvasion i.e. <1cm (11.7%) (Figure 5). Studies shows higher the tumour stage less pronounced is the intraductal component.

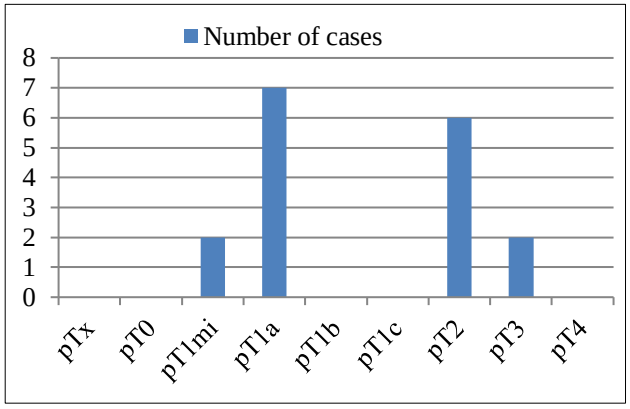

Figure 5: Tumour size distribution.

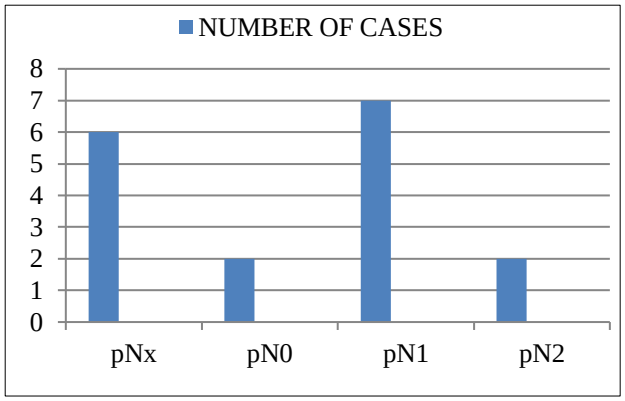

Figure 6: Nodal involvement distribution

Pathological node involvement was also calculated out of which 7 cases (41.1%) had pN1 nodal involvement followed by 6 cases (35.2%) in pNx and the least involved pathological nodes were p No and pN2 each having 2 cases (Figure 6) (2+2) (11.76%).

Distribution of ER/PR/Her2neu/Ki67

Estrogen receptor (ER) and progesterone receptor (PR) are hormone receptors found on breast cells that pick up hormone signals resulting in cell growth. Similarly, positive human epidermal growth factor receptor-2 (HER2/neu) status of the breast carcinoma means that HER2/neu gene is making too many HER2/neu proteins, which acts as receptors on the cell surface and helps the cells to grow and divide. Hormone receptor studies such as ER, PR, and HER2/neu are routinely done in breast carcinoma. It not only helps in the prognosis of the tumor but also helps in deciding its treatment. IHC for hormone receptor expression was done.6 cases had ER+ /PR+/HER2NEU-(35.2%). Ki67 was done for each of the cases where 4 cases had high Ki67 out of 6 cases of ER+/PR+/HER2NEU- (figure7). In the figure 7 we have the following interpretation –ER+/PR+/HER2NEU- (6 cases), ER+/PR+/HER2NEU+(3cases), ER-/PR- /HER2NEU+(2cases), ER-/PR/HER2NEU(4cases), ER+/PR-/HER2NEU-(2cases).

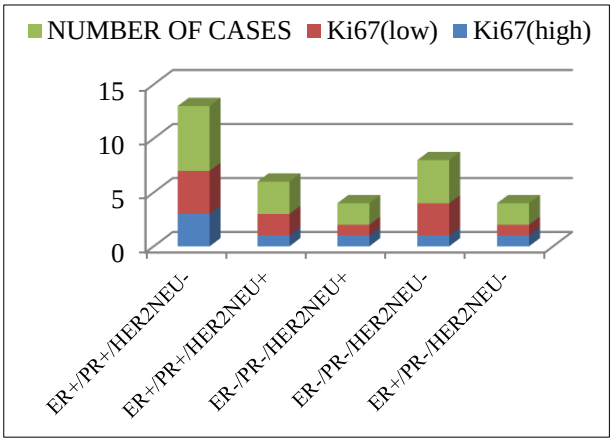

Figure 7: ER/PR/HER2NEU and Ki67 distribution.

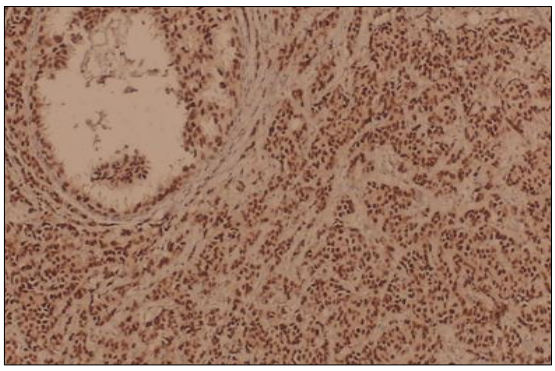

Figure 8: ER positivity in invasive carcinoma.

Breast cancer is called estrogen receptor positive (ER+) if it has receptor for estrogen. Figure 8 below shows the immunohistochemical studies detected proteins using

specific antibodies visualized by a brown chromogen which is the ER-positive receptor.The estrogen receptors mainly takes the nuclear stain, which shows the invasive component of the breast cancer,where the tumour cells take more than >80% of the nuclear stain for ER receptors which is seen the figure 8. The score as already mentioned is determined by ALLRED SCORING system.

Figure 9 showing an immunostained section of invasive breast carcinoma with>70% positivity for PR receptors. In the figure 9 the immunostained section of the invasive breast cancer mainly detects the protein for PR receptor which is indicated by a brown chromogen.PR receptor mainly takes the nuclear stain which is clearly shown in the figure 9. The presence of the hormone receptors ER, PR in a patient's breast cancer is an example of a weak prognostic but strong predictive biomarker. If a patient's tumor expresses ER and/or PR, we can predict that this patient will positively benefit from endocrine therapy such as tamoxifen.

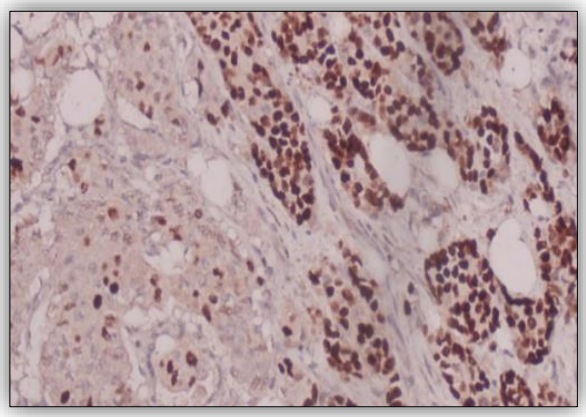

Figure 9: PR+ in invasive breast cancer.

HER2 protein overexpression is detected in the tumor cells by immunohistochemical stain which is shown in the figure 10.

In the figure 10 it is also seen that the Her2neu is a cytoplasmic stain which mainly stains the cytoplasm of the tumour cells in the invasive carcinoma of the breast. The tumour cell displays complete intense circumferential membranous staining in >10% of the tumour cells which shows a strong IHC reaction (IHC 3+) shown in the figure 10.

Normally, HER2/neu receptors control healthy breast cell growth, division, and repair. However, in about 30% of breast cancers, the HER2/neu gene does not work correctly and makes too many copies of it (known as HER2/neu gene amplification).

These extra HER2/neu gene copies tell breast cells to make too many HER2/neu receptors (HER2/neu protein overexpression). This ultimately makes breast cells to grow and divide in an uncontrolled way.

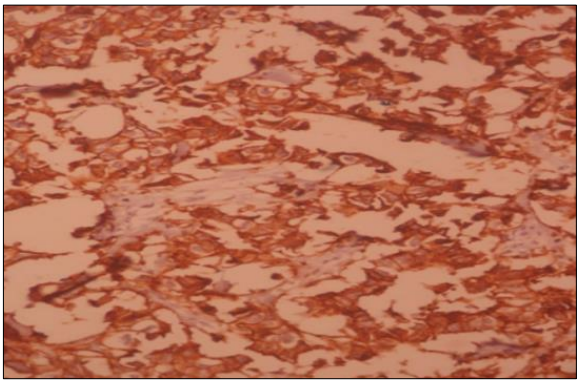

Figure 10: HER2NEU positivity case in invasive breast cancer.

Ki-67 protein in humans is encoded by MK167 gene and is a cellular marker for proliferation. This is a nuclear protein and is expressed in proliferating cells but is not detected in resting cells. The Ki-67 expression as detected by immunohistochemistry is one of the most reliable indicators of the proliferative status of cancer cells.Figure 11 shows Ki67 positivity in an invasive breast carcinoma, which takes the nuclear stain. In the figure 11 the immunostained section of the breast tissue shows the grade of Ki67 stain pattern is mainly of high grade with >10% of the cells showing Ki67 nuclear stain.

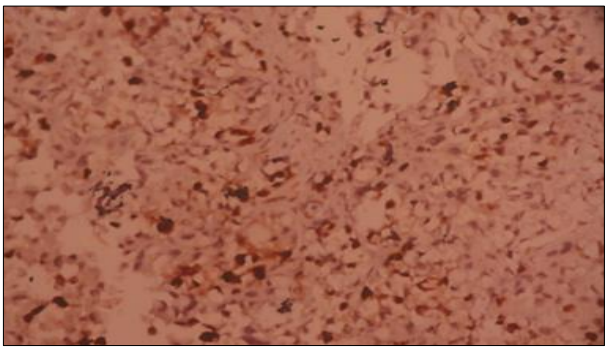

Figure 11: High KI67 positivity in invasive breast cancer.

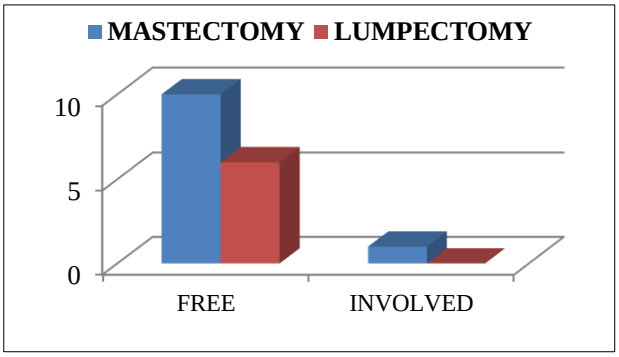

Figure 12: Types of surgery and margin status.

Most of the cases were mastectomy cases 11(64.4%) with a base free status except in one mastectomy case where margin was involved. The margins of every specimens of breast conservative surgery were marked in different dimensions (medial, lateral, superior, inferior) at the time of the surgery (Figure 12).

DISCUSSION

In this study we evaluated the presence of EIDC in the biopsied cases suspected for breast carcinoma where we have analyzed 17 cases of EIDC out of 65 cases of invasive carcinoma.

Various parametres have been in studied in relation to EIDC prevelance.

The age group in the current study ranged from 27-73 years with mean age of 43 years and 5 patients were in postmenopausal stage which was in accordance with study done by Noushad et al where mean age was 48.15 years,and Christopher et al, Jacquemier et al with a mean age of 46.8 years.[7,8]

Based on IHC our cases were classified according to the ER/PR/HER2NEU status out of which 6(35.2%) cases were ER+/PR+/HER2NEU-.

Ki67 was high in most of the cases which was 4 cases out of 6 in the majority group of ER+/PR+/HER2NEU-.

This study had similar results to the study done by Christopher et al and Carla et al but study by Noushad et al showed the majority of cases in ER-/PR-/HER2NEU+.[7,9]

In the current study we have found most of the cases falls in grade II (82.3%) of BRG followed by grade I (17.1%), with similar results found out by Carol et al-Grade II (50.8%).

This shows that most of the EIDC cases falls in lower grade.

pT and pN distribution in EIDC showed that most of the cases to be in pT1 and pN1, which was in accordance to the study done by Noushad et al, (pT1a-58%).[3]

Considering the current study and most of the study done earlier it has been observed that higher percentage of EIDC corresponds to pT1.

This fact suggest that the invasive tumour that measured between 1 to 2cm exhibits a higher probability to be associated with EIDC, unlike lesions in more advanced stages, i.e. stage pT4,where the component has already acquired earlier in situ profile and is less often found in these lesions.[3]

Studies showed that EIDC is associated with high local recuurence rate. So evaluation of EIDC, along with the negative margin status is important to prevent recurrence.

In the current study most of the cases whether mastectomy or lumpectomy, the margins were free of tumour foci except in one case of mastectomy where the margin was involved.

Study done by Holland et al, Schnitt et al, showed that 71% of cases and 88% of cases with positive EIC in CNB were associated with residual DCIS in subsequent mastectomy cases.[10-12]

It suggests that the distribution of EIC associated with tumors has been underestimated in their contribution to surgical planning.

CONCLUSION

Extensive intraductal component (EIC)positive carcinoma of breast is an uncommon entity most commonly found in premenopausal age group, with usually low pathological stage, lower rate of lymphnode metastasis, ER, PR positivity and Her2Neu negative status although it is associated with an extensive component.

This study mainly focused on EIDC prevelance and expression of ER/PR/HER2NEU/Ki67.

As these biomarkers will not only help in prognosis of the disease but will also help in the prediction of the disease ultimately helping in selecting the right treatment regimen for the patients.

Resected margin clearance status is always a concern in EIDC as it is highly associated with local recurrence. Prognosis of this new entity i.e. EIDC is good if adequate margin clearance is attained during surgery.

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

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