Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

EVALUATION OF E-CADHERIN EXPRESSION IN INVASIVE BREAST CARCINOMA AND ITS HISTO-PATHOLOGICAL CORRELATION

Dr.Biswajit Padhy[1],Dr.Saroj Ranjan Mohanty[2],Dr.Soumya Ranjan Jena [3],

Dr.Ambuja Satapathy [4] ,Dr.Subhakanta Das[5]

[1]Insurance Medical Officer(Grade-1),Rourkela,Odisha

[2]Assistant professor, Department of Pathology, MKCG Medical College, Berhampur, Odisha

[3]Assistant professor, Department of General Surgery, MKCG Medical College, Berhampur, Odisha

[4] Associate Professor, Department of General Surgery, MKCG Medical College, Berhampur, Odisha

[5] Assistant Professor, Department of Pathology, MKCG Medical College, Berhampur, Odisha

Corresponding Author : Dr.Ambuja Satapathy, Associate Professor, Department of General Surgery, MKCG Medical College, Berhampur, Odisha

ABSTRACT

BACKGROUND:

Breast carcinoma is the most common malignant tumour and the second most common cause of carcinoma death in women. In the present scenario predicting disease outcome is becoming increasingly important, especially for planning treatment strategies and for counselling the patient. Numerous prognostic markers have also been identified in recent years through increasing understanding of the biology of breast cancer. One group of these possible factors is the Cadherin family. Selective loss of E-Cadherin can cause de-differentiation and invasiveness in human carcinomas. Reduced expression of E-Cadherin has been observed in aggressive tumours of the oesophagus, ovary, and stomach. These data indicate that E-Cadherin is a potential bio-marker to predict which patients will experience more aggressive form of the disease. This information can potentially be used to reduce morbidity and mortality in breast cancer patients. Hence this study was undertaken to find out the expression of E-Cadherin in invasive breast cancers by IHC assay along with other routine IHC markers like ER, PR, and HER- 2/neu, as well as to correlate the expression of E-Cadherin with tumour aggressiveness in terms of different parameters like histopathologic type, grade, lymph node status, tumour size and molecular subtypes.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

AIM AND OBJECTIVE:

- To study the expression of E-Cadherin in invasive breast cancers by IHC assay along with other routine IHC markers like ER, PR, and HER- 2/neu.
- To correlate the expression of E-Cadherin with tumour aggressiveness in terms of different parameters like
  - Histopathologic type
  - Histologic grade
  - Lymph node status
  - Tumour size
  - Molecular subtypes
- As an aid to the sub-classification of invasive breast cancer.

PATIENTS AND METHODS:

This prospective study was conducted in the Department of Pathology at **M.K.C.G. MEDICAL COLLEGE AND HOSPITAL, BERHAMPUR** from the period of September 2018 to August 2020, with approval of ethical committee of this institute. All histologically (core needle biopsy) proven cases of invasive carcinoma of breast irrespective of age were included in the study. The patients were undergone surgery as per standard treatment protocol and specimens were collected for IHC and histopathological study. Total 76 number of patients were included in this study. The detail data were recorded as per the predefined proforma.

RESULTS:

Maximum patients were above 40 years of age and were pre-menopausal (80% and 55%, respectively). IDC NOS was the major histologic type constituting 87% of total. Other types found were Lobular (5%), Medullary (4%), Metaplastic (3%), and Mucinous (1%) variants.36 out of 76 cases (48%) were of size > 2 cm to ≤ 5 cm (T2 stage).Lymph node metastasis was present in 39 cases and maximum were in N1 stage (26%).Histologic Grade III was the most common (54%) followed by Grade II (37%) and Grade I (9%).Most common molecular subtype was Luminal A (50%) followed by Basal Like/Triple Negative (28%), Luminal B (17%) and HER2 enriched (5%).E-Cadherin was found to be negative in 35 out of 76 cases (46%).Higher histologic grade and lymph node stage had decreased E-Cad activity. E-Cad expression was completely lost in Her2/neu enriched (100%) while maximally lost in Basal like/triple negative and Luminal B phenotypes (62% in each type). Loss of E-Cadherin expression in breast cancer correlates with Menstrual status, higher histologic grade, positive lymph node status and specific molecular subtypes (TNBC and Her2 enriched).

CONCLUSION:

In the present study we specifically determined the expression of E-Cadherin protein in primary invasive breast carcinomas and their histopathological correlation. Diminished E-Cadherin Expression in higher grades and positive lymph node cases supports the view that loss of E-Cadherin expression is a marker of aggressiveness. Further, E-Cadherin can be a useful bio marker to differentiate between IDC and ILC since all Lobular carcinomas were negative for E-Cadherin expression. However, since there were only

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

four cases of ILC, large scale studies are needed to establish this difference and to confirm the prognostic value of E-Cadherin.

INTRODUCTION:

Breast carcinoma is the most common malignant tumour and the second most common cause of carcinoma death in women, with more than 1.7 million cases occurring worldwide annually [1]. Despite massive advances in detection and treatment, it continues to be a major cause of morbidity and mortality throughout the world. According to latest world cancer statistics published by the International Agency for Research on Cancer (IARC) in 2018, breast cancer represents 11.6% of diagnosed cancers [2]. Breast cancer is also the leading cause of cancer death among women (15%) and accounts for approximately 5, 20,000 deaths per year. Since 2008, the incidence of breast cancer has increased by 20%, while mortality has increased by 14% [3]. There are different approaches to the classification of breast cancer. Traditionally, histopathologic features have been used to classify breast carcinomas. However, more recent classifications categorize the disease at the molecular level, since it gives important predictive information on the potential responsiveness of the tumours to different therapeutic modalities. In the present scenario predicting disease outcome is becoming increasingly important, especially for planning treatment strategies and for counselling the patient. Assessment of certain prognostic 'markers' or 'factors' is now routine in the pathological examination of breast tumours, in order to give an indication of suitability of certain forms of treatment, the risk of recurrence and expected length of survival. Some of the more traditional markers include histological typing and grading, primary tumour size, lymph node stage, vascular invasion, and excision margin status. Numerous prognostic markers have also been identified in recent years through increasing understanding of the biology of breast cancer. One group of these possible factors is the Cadherin family.

E-Cadherin is a calcium regulated adhesion molecule expressed in most normal epithelial tissues. The E-Cadherin gene is located on chromosome 16q.22.1 [4]. It is associated with gland formation, stratification, and epithelial polarization [5]. Selective loss of E-Cadherin can cause de-differentiation and invasiveness in human carcinomas. In various cell lines, a reciprocal relationship has been shown between levels of E-Cadherin expression and invasiveness [6]. Reduced expression of E-Cadherin has been observed in aggressive tumours of the oesophagus, ovary, and stomach [7-9]. Mechanisms by which E-Cadherin protein expression is lost include gene mutation and loss of the wild type allele by loss of heterozygosity [10-12]. These data indicate that E-Cadherin is a potential bio-marker to predict which patients will experience more aggressive form of the disease. This information can potentially be used to reduce morbidity and mortality in breast cancer patients. Hence this study was undertaken in the Department of Pathology, M.K.C.G. Medical College to study the expression of E-Cadherin in invasive breast cancers by IHC assay along with other routine IHC markers like ER, PR and HER- 2/neu, as well as to correlate the expression of E-Cadherin with tumour aggressiveness in terms of different parameters like histopathologic type, grade, lymph node status, tumour size and molecular subtypes.

AIM AND OBJECTIVE OF THE STUDY:

- To study the expression of E-Cadherin in invasive breast cancers by IHC assay along with other routine IHC markers like ER, PR, and HER- 2/neu.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

- To correlate the expression of E-Cadherin with tumour aggressiveness in terms of different parameters like
  - Histopathologic type
  - Histologic grade
  - Lymph node status
  - Tumour size
  - Molecular subtypes
- As an aid to the sub-classification of invasive breast cancer.

MATERIALS:

This prospective study was conducted in the Department of Pathology at **M.K.C.G. MEDICAL COLLEGE AND HOSPITAL, BERHAMPUR** from the period of September 2018 to August 2020, with approval of ethical committee of this institute. All histologically (core needle biopsy) proven cases of invasive carcinoma of breast irrespective of age were included in the study. The patients were undergone surgery as per standard treatment protocol and specimens were collected for IHC and histopathological study. Total 76 number of patients were included in this study. The detail data were recorded as per the predefined proforma.

Inclusion criteria:

All histologically proven cases of invasive carcinoma of breast irrespective of age were included in the study.

Exclusion Criteria:

1) Cases where only a tru-cut biopsy or limited surgery has been done (all parameters were not available).
2) Cases where there was extensive tumour necrosis without sufficient viable tumour cells.

METHODS:

After that, gross details of the specimen were recorded with reference to size, colour, ulceration, consistency, involvement of nipple & areola, presence of necrosis & haemorrhage. Lymph nodes, if present their number, size & cut section features were noted. Then microscopic study of the specimen was done with the help of expert pathologists, and lastly Immunohistochemical staining using ER, PR, Her2/neu, and E-Cadherin was performed.

Histological grading and typing: Cancers were graded according to the Nottingham's combined histologic grade (Elston-Ellis' modification of Scarff-Bloom-Richardson's original classification from 1957) [13] and Tumour typing was performed according to WHO classification 2019 [14].

Immunohistochemical studies: Paraffin blocks which were most representative of tumour tissue were chosen for performing immunohistochemistry. It was done for evaluation of oestrogen and progesterone

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

receptors, Her-2/neu, and E-Cad status. Dako primary antibodies were utilized for this purpose. The expression of E-Cad was first studied in the tumours following which it was compared with the respective histological grading, Tumour size, lymph node status, hormone receptor, HER2/neu status, and molecular sub-types etc.

TISSUE PROCESSING TECHNIQUES (Bancroft) [15]:

Tissue sections were processed in auto processor for preparation of paraffin blocks as per the following schedule. Prior to processing, specimens were fixed in 10% **Neutral Buffered Formalin (NBF)** within 30 minutes of surgery & for not more than 48 hours (ideal is 12-24 hours). The fixative used preserve the antigenic integrity & limit the extraction, diffusion, or displacement of antigen during subsequent processing.

STEPS OF PROCESSING:

Fixation in 10% NBF	-	4hours
Fixation in 10% NBF	-	4hours
Dehydration in 50 % isopropyl alcohol	-	1hour
Dehydration in 70 % isopropyl alcohol	-	1hour
Dehydration in 90% isopropyl alcohol	-	1hour
Dehydration in absolute alcohol	-	1hour
Dehydration in absolute alcohol	-	1hour
Dehydration in absolute alcohol	-	1hour
Clearing in xylene	-	1hour
Clearing in xylene	-	1hour
Paraffin impregnation in first bath	-	2hours at 65^0c
Paraffin impregnation in second bath	-	2hours at 65^0c

PREPARATION OF BLOCK:

After paraffin impregnation, blocks were prepared by transferring them from the final wax bath to a mould (L-piece) filled with molten wax. The blocks were then quickly cooled.

SECTION CUTTING:

The blocks were kept over ice prior to cutting then it was fixed to a block holder and 4-5µ thin sections were cut by rotator microtome. The sections were fixed to the glass slides coated with Mayer's egg albumin.

STAINING:

Haematoxylin and Eosin staining (Bancroft) [15]

Deparaffinization by slight heating	-	2-3mins
Xylene	-	2-3mins
Xylene	-	2-3mins
Absolute alcohol	-	1min
95% alcohol	-	1min
70% alcohol	-	1min
Rinsing in running water	-	5min
Harris Haematoxylin staining	-	2-4mins
Quick rinse in tap water		
Differentiation in 1% acid alcohol	-	1-2dips

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

- Rinse in tap water
- Blue in 0.2% ammonia water
- Rinse well in running tap water - 10min
- Stained in 1%aqueous Eosin - 1min
- 80% ethyl alcohol - 1min
- 90% alcohol - 1min
- Absolute alcohol - 1min
- Dry /blot the slides
- Xylene 2 changes
- Mounting using DPX

Results:

Nucleus : Stained blue
Cytoplasm : Stained pink

REAGENTS & EQUIPMENTS FOR IHC:

Primary antibodies **(Table 1)**

1. Polymer detection system
2. Positively charged slides (AMA45-Color Microscope charged slides)
3. Conventional microwave oven/pressure cooker
4. Moist chamber
5. Electronic weighing machine
6. pH indicator strips
7. Thermoresistant plastic slide container
8. Micropipette
9. Chemicals- Tris (Hydroxymethylaminomethane), Disodium EDTA, Sodiumdihydrogen orthophosphate, Disodium hydrogen orthophosphate and Sodium chloride.
10. 6% Hydrogen Peroxide, Xylene, isopropylalcohol, deionized water & glass beakers.

TABLE (1): Primary antibodies used for IHC

Antigen	Antibody	Clone	Lab	Dilutions
ER alpha	Rabbit monoclonal	EP1	DAKO	Ready to use
PR alpha	Mouse monoclonal	636	DAKO	Ready to use
HER2/neu	Rabbit polyclonal	EP3	BioGenex	1:500
E-Cadherin	Mouse monoclonal	NCH 38	DAKO	1:50

Dilution of Her2neu (1:500)

2µL of cerbB-2 antibody + 1ml of wash buffer for preparation of 1ml solution.

Dilution of E-Cadherin (1:50)

20µL of E-Cad antibody + 1ml of antibody diluent for preparation of 1ml solution.

IMMUNOHISTOCHEMISTRY [15-16]

Preparation for Immunohistochemistry:

- Charged slides were taken (AMA45-Color Microscope charged slides)
- Measuring cylinders & staining jars were washed with distilled water & dried before use.
- pH papers ranging from 3-10 are kept ready.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

- **Antigen - Retrieval buffer:** Dako EnVision™ Flex target retrieval solution high Ph (50X). The dilution was 1: 50 in distilled water and pH was 9.
- **Wash Buffer:** Dako EnVision™ Flex wash buffer (20x) was used for all cases. The dilution of wash buffer was 1:20 in distilled water.
- **Hydrogen Peroxide:** Ready to use Dako EnVision™ Flex peroxidase blocking agent was used.
- **Preparation of primary antibodies** -DAKO monoclonal mouse anti-human E-Cadherin clone NCH-38 in 1:50 dilution in distilled water was used.
- **Preparation of secondary antibodies:** Ready to use Dako EnVision™ Flex / HRP was used.
- **Preparation of DAB solution:** Dako EnVision™ Flex DAB+ chromogen was used. DAB solution was freshly prepared by adding one drop of DAB in 1 ml of Dako EnVision™ Flex substrate buffer– in black mixing vial. It was mixed in figure of 8 fashion by keep on a flat surface.

IHC PROCEDURE [15-16]

1. **Mounting paraffin sections onto slides**
   - **a.** Thin sections were cut(3-4µ)
   - **b.** The sections were mounted on to charged slides
   - **c.** The slides were labelled and dried in upright position overnight
   - **d.** The slides were deparaffinised on hot plate 60^0C for 1 hour
2. **Rehydration of slides**
   - **a.** 2 changes of Xylene 5 min each
   - **b.** Graded alcohol
     - **i.** 100% Alcohol 5 min
     - **ii.** 90% Alcohol 5 min
     - **iii.** 70% Alcohol 5 min
     - **iv.** 50% Alcohol 5 min
   - **c.** Distilled water 30 sec or till next step.
3. **Heat induced Antigen retrieval**
   - **a.** Tissue sections were placed in a container with antigen retrieval solution.
   - **b.** This container was placed in a domestic pressure cooker with water.
   - **c.** Boiled over low flame till first whistle.
   - **d.** Heat was turned off on first whistle, allowed to cool by itself and opened after complete cooling.
4. **The slides were taken out of the antigen retrieval solution and put in wash buffer till next step.**
   - **a.** Extra care was taken to prevent drying of the tissues on the slide at any step during the procedure. The slides were kept in wash buffer to prevent drying.
   - **b.** Supplied Wash buffer: Distilled water – 1:19 proportion
   - **c.** The slides were rinsed in wash buffer to remove excess antigen retrieval solution (5 min)
5. **Next steps were done in humidity chamber – slide box with wet cotton paced in the bottom**
6. **Peroxide block**
   - **a.** The slides were taken out of wash buffer and wiped around the sections and placed in humidity chamber
   - **b.** 1 drop of peroxidase blocking agent was placed over each tissue sections
   - **c.** Incubated for 10 minutes closed in the humidity chamber

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

d. The slides were removed from chamber and dipped in wash buffer to remove peroxide excess – 1 min / 10 dips

7. **Primary antibody (CD marker) – specific for each tissue section**
   - **a.** The slides were taken out of wash buffer, wiped around the sections and placed in humidity chamber.
   - **b.** Specific primary antibody (DAKO monoclonal mouse anti-human E-Cadherin clone NCH-38) was placed on each tissue section – quantity was just enough to cover the tissue section.
   - **c.** Incubated for 30 minutes closed in the humidity chamber.
   - **d.** The slides were removed from chamber and dipped in wash buffer to remove excess primary antibody – 1 min / 10 dips.
8. **Secondary antibody / Enzyme marker – Horse Radish Peroxidase (HRP)**
   - **a.** The slides were taken out of wash buffer, wiped around the sections and placed in humidity chamber.
   - **b.** HRP was placed on each tissue section – quantity was just enough to cover the tissue section.
   - **c.** Incubated for 30 min closed in the humidity chamber.
   - **d.** The slides were removed from chamber and dipped in wash buffer to remove excess HRP – 1 min / 10 dips.
9. **Chromogen - DiAminoBenzidine (DAB)**
   - **a.** DAB was freshly prepared.
   - **b.** The slides were taken out of wash buffer, wiped around the sections and placed in humidity chamber.
   - **c.** Freshly prepared DAB was placed on each tissue section – quantity was just enough to cover the tissue section.
   - **d.** Incubated for 5 min / till brown colour appears in the humidity chamber.
   - **e.** The slides were removed from chamber and thoroughly rinsed in distilled water to remove excess DAB
10. **Counterstaining**
    - **a.** The slides were dipped in haematoxylin for 1min
    - **b.** Washed in running tap water for bluing – 5 min
11. **Dehydration**
    - **a.** Graded alcohol
      - **i.** 50% Alcohol 10 min
      - **ii.** 70% Alcohol 10 min
      - **iii.** 90% Alcohol 10 min
      - **iv.** 100% Alcohol 10 min
12. **Mounting was done using DPX**

Precautions:

1. At any stage the sections should not be allowed to dry.
2. Steps of incubation should be carried out with Ab at 37^0c in humid chamber.
3. The solutions used for deparaffinisation should be changed after 2-3 times of use.
4. The alcohols should be changed periodically.
5. DAB is a carcinogen, so should be handled carefully.
6. Appropriate positive and negative controls were used for each antibody.

INTERPRETATION OF RESULTS:

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

For all the markers, only the invasive component of the tumour was considered.

E-Cadherin: Immunostains were evaluated independently, and any differences in interpretation were resolved by simultaneous viewing. Only the membrane staining intensity and pattern was evaluated on a scale of 0 to 3. Scores 0 and 1+ were considered negative. Immunoreactivity of 2+ and 3+ was scored as positive. Cytoplasmic staining was rare, considered nonspecific, and not included in assessment. The presence of EC staining in epithelial cells of normal ducts and acini served as an internal positive control in every case. (**Qureshi et al /Am J Clin Pathol 2006)** [17] (**Table: 2)**

TABLE (2): E-Cadherin Scoring System

Score	Cell Membrane staining pattern	Interpretation
0	No staining or membrane staining in <10% of tumour	Negative
1+	Faint incomplete membrane staining in >10% of tumour	Negative
2+	Weak or moderate complete membrane staining in >10% of tumour	Weakly Positive
3+	Strong complete membrane staining in >10% of tumour	Strongly Positive

Result:

≥ 10% Cell membrane staining: - Positive (score 2-3)

< 10% Cell membrane staining: - Negative (score 0-1)

Estrogen and Progesterone receptor (ER/PR):

Currently there is no single recommended system worldwide. A simple method known as **'Allred/Quick score' (Table: 3)** system which considers the summation of the proportion of tumour cells showing reactivity and intensity of staining [18].

TABLE (3): Quick Score for ER &PR

Proportion score	Intensity score
0= No staining. 1= <1% nuclear staining. 2= 1-10% nuclei staining. 3= 11-33% nuclei staining. 4= 34-66% nuclei staining. 5= 67-100% nuclei staining.	0= No staining 1= Weak staining. 2= Moderate staining. 3= Strong staining.

Final score is obtained by adding scores from the 2 categories to give a maximum score of 8. Tumours with score < 3 are termed negative while those with score ≥ 3 are termed positive.

HER-2/neu: (ASCO and CAP guidelines 2013) [19] **(Table: 4)**

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

TABLE (4): HER2/neu scoring system

Staining pattern	Score	Assessment
No staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumour cells.	0	Negative
Incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumour cells.	1+	Negative
Circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of the invasive tumour cells; or complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumour cells.	2+	Weakly positive/ Equivocal
Circumferential membrane staining that is complete, intense in > 10% invasive tumour cells.	3+	Positive

N.B: In case of 2+ score (equivocal), the test is further subjected to FISH for confirmation. As there is no facility for FISH in our set up, 2+ score was considered to be positive.

QUALITY CONTROL:

Differences in tissue processing & technical procedure may produce variable results. Hence controls used are fresh surgical specimens processed in same manner as patient's sample. The control slides were provided by Dako manufacturers.

POSITIVE TISSUE CONTROL:

Positive control was used to indicate correctly prepared tissues & proper staining.

NEGATIVE TISSUE CONTROL:

Negative control was used to verify the specificity of the labelling of the target Ag by primary Ab. Example-primary antibody was not added in the procedure.

TROUBLE SHOOTING:

It is a multistep diagnostic procedure involving proper selection, fixation, processing & staining of tissues.

1. Problems may be prior to staining & during it.
2. False Negative staining (FNS)
3. False Positive staining (FPS)

FALSE NEGATIVE STAINING:

1. Incomplete deparaffinization
2. Incorrect retrieval solution
3. Inadequate retrieval temperature
4. Extreme digestion in enzyme retrieval
5. Extreme degree of atmospheric temperature
6. Abnormal Concentration
7. Chromogen incompatibility

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

8. Positive control selection
9. Process failure

FALSE POSITIVE STAINING:

1. Poor quality of fixation.
2. Poor technical preparation of tissues.
3. Defective pre-treatment.
4. Over digestion using enzyme retrieval.
5. High temperature oven exposure.
6. Intrinsic tissue biotin.
7. Blocking (peroxidase, protein).
8. Abnormal concentration.
9. Detection system.
10. Species cross reactivity.
11. Tissue drying.
12. Automation error.

STASTICAL METHOD:

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean ± SD (min- max) and results on categorical measurement are presented in number (%).

Significant figures of P- value:

- Suggestive significance (P-value: $0.05 < P < 0.10$)
- Moderate significance (P-value: $0.01 < P \leq 0.05$)
- Strong significance (P-value: $P \leq 0.01$)

For all such categorical data Chi-Square Test was applied using IBM SPSS Statistics 20. P-value < 0.05 was considered as the minimum level of significance.

RESULTS AND DATA ANALYSIS:

AGE DISTRIBUTION:

TABLE (5): Distribution of cases according to age (n=%)

Age in years	Frequency	Percentage %
< 40	15	20
≥ 40	61	80
Total	76	100

Shows distribution of breast carcinoma patients according to 2 different age groups. A total of 76 mastectomy specimens of Breast carcinoma cases were taken for histopathological examination followed by immunohistochemical assay. 80% of them (61 out of 76 cases) were in ≥ 40 years of age group. The mean age of the patients was worked out to be 50 years with a standard deviation of 12.08.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

DISTRIBUTION ACCORDING TO MENSTRUAL STATUS

TABLE (6): Distribution of cases according to menstrual status (n= %)

Menstrual Status	Frequency	Percentage %
Pre-Menopausal	42	55
Post-Menopausal	34	45
Total	76	100

Shows distribution of breast cancer patients according to their menstrual status. Accordingly, they are divided into pre / post-menopausal group. The menstrual status has been collected through clinical history of the patients. Out of 76 breast cancer patients, 55 % (42 cases) were in pre-menopausal group and rest 45 % (34 cases) were in post-menopausal group.

DISTRIBUTION ACCORDING TO HISTOLOGIC TYPES

TABLE (7): Frequency distribution of different histological variants of carcinoma breast (n= %)

Histological Subtype	Frequency	Percentage %
IDC NOS	66	87
Lobular	04	5
Medullary	03	4
Metaplastic	2	3
Mucinous	1	1
Total	76	100

Shows frequency distribution of different histological variants in terms of proportion. IDC-NOS with 87% were the most common variant followed by Lobular carcinoma (5%) and Medullary Carcinoma (4%). Metaplastic and Mucinous variants were very few comprising 3% and 1% respectively.

DISTRIBUTION ACCORDING TO TUMOUR SIZE (T STAGE):

TABLE (8): Distribution of cases according to tumour size (n= %)

Tumour Size in cm	Frequency	Percentage %
T1 (≤ 2)	13	17
T2 (> 2 to ≤5)	36	48
T3 (>5)	26	34
T4 (any T with chest wall/ skin involvement)	1	1
Total	76	100

Shows distribution of tumours according to the size or T-stage. T2 variant was found to be most common with 48% (36 cases), whereas T4 tumours were least in number with only 1% (1 Case). Rest 2 variants i.e., T1 and T3 were distributed as 17% (13 Cases) and 34% (26 Cases) respectively.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

DISTRIBUTION ACCORDING TO LYMPH NODE METASTASIS

TABLE (9): Distribution of cases according to lymph node status (n= %)

Lymph Node Stage	Frequency	Percentage %
N0	37	49
N1	20	26
N2	16	21
N3	3	4
Total	76	100

Shows distribution of breast cancer cases according to nodal metastasis. Maximum 37 cases (49%) were not having lymph node metastasis, followed by N1 metastasis (20 cases, 26%) and N2 metastasis (16 cases, 21%). Only 3 cases (4%) were having N3 metastasis.

DISTRIBUTION ACCORDING TO HISTOLOGICAL GRADE

TABLE (10): Distribution of different histological grades (n=%)

Histological Grade	Frequency	Percentage %
Grade 1	7	9
Grade 2	28	37
Grade 3	41	54
Total	76	100

Shows distribution of mastectomy specimens according to Histological grade. Out of 76 breast specimen samples, majority (54% i.e., 41 cases) were found to be Grade-III tumour. Grade-II tumours were 37% (28 cases) and only 9% (7 cases) were Grade-I.

DISTRIBUTION ACCORDING TO MOLECULAR SUBTYPES

TABLE (11): Distribution of different molecular subtypes (n= %)

Molecular Subtype	Frequency	Percentage %
Luminal A	38	50
Luminal B	13	17
HER 2 Enriched	4	5
Basal Like	21	28
Total	76	100

Shows distribution of breast cancer cases according to molecular subtypes. Exactly Half of the molecular subtypes, 38 cases out of 76 (50%) were found to be Luminal A, 21cases (28%) were Basal Like type, 13 cases (17%) were Luminal B, and only 4 cases (5%) were of HER2 enriched variant.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

DISTRIBUTION OF HISTOLOGICAL GRADES IN DIFFERENT AGE GROUPS:

TABLE (12): Histological grades in different age groups (n=76)

Age groups	Histological Grade			
	Grade-I	**Grade-II**	**Grade-III**	**Total**
< 40	1	8	6	**15**
≥ 40	6	20	35	**61**
Total	**7**	**28**	**41**	**76**

Shows distribution of different histological grades in different age groups. Grade II tumours were found to be more frequent (53%) in < 40 groups. Whereas Grade III tumours were found to be common in ≥ 40 age groups (57%).

HORMONAL RECEPTOR STATUS:

TABLE (13): Hormonal receptor status (n=76)

HRS	**ER**	**%**	**PR**	**%**	**HER 2/neu**	**%**
Positive	51	67	51	67	16	21
Negative	25	33	25	33	60	79
Total	76	100	76	100	76	100

Shows distribution of breast cancer cases according to hormone receptor status. Out of 76 cases, 51 cases (67%) were found to be equally positive for both ER and PR whereas 16 cases (21%) were Her2/neu Positive.

E-CADHERIN STATUS:

TABLE (14): E-Cadherin status in terms of membrane staining (n= %)

E-Cadherin Status	**Frequency**	**Percentage %**
Positive (2+/3+)	41	54
Negative (0/1+)	35	46
Total	76	100

Shows distribution of breast cancer cases according to E-Cadherin status. Out of 76 cases 41 (54%) were E-Cad Positive and 35 (46%) were E-Cad Negative.

E-CADHERIN STATUS IN RELATION TO AGE:

TABLE (15): E-Cad in relation to different age groups (n= %)

E-Cad Expression (In terms of membrane staining)	**Age groups**			**Statistics**
	< 40 Years	≥ 40 Years	Total	Chi-square value= **0.0037,**

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

Positive (2+/3+)	8	32	40	Dof= 1 p-value= **0.951552**
Negative (0/1+)	7	29	36	
Total	15	61	76	

Shows E-Cadherin status in relation to different age groups. E-Cadherin was Negative in 46% of cases (7 out of 15) among < 40 age group. Among ≥ 40 years age group 48% tumours (29 out of 61) were E-Cadherin Negative.

E-CADHERIN STATUS IN RELATION TO MENSTRUAL STATUS:

TABLE (16): E-Cad in relation to menstrual status (n= 76)

E-Cad expression (in terms of membrane staining)	**Menstrual status**			**Statistics**
	Pre-Menopausal	Post-Menopausal	Total	
Negative (0/1+)	27	10	37	Chi-square value = **9.147,** Dof=**1,** p-value= **0.0024**
Positive (2+/3+)	15	24	39	
Total	42	34	76	

Shows E-Cadherin in relation to the menstrual status of women. Among 42 premenopausal breast cancer patients E-Cad was Negative in 64% of the cases. Similarly, 30% of 34 post-menopausal breast cancer patients were found to be E-Cad Negative. It was found that loss of E-Cadherin expression had got close significant association with menstrual status. (p- value < 0.05).

E-CADHERIN STATUS IN RELATION TO HISTOLOGICAL TYPES:

TABLE (17): E-Cad status in relation to histological types. (n=76)

E-Cad expression (In terms of membrane staining)	**Histological Sub-types**						**Statistics**
	IDC NOS	LOBULAR	MEDULLARY	METAPLASTIC	MUCINOUS	Total TOTAL	
Negative (0/1+)	29	4	1	1	0	35	Chi-square value=**5.866** Dof=**4,** p-value= **0.209**
Positive (2+/3+)	37	0	2	1	1	41	
Total	66	4	3	2	1	76	

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

Shows E-Cadherin status in different Histological subtypes. E-Cad was found to be negative in all four cases (100%) of Lobular carcinoma, 44% cases were found to be negative in IDC NOS type, 33% of cases in Medullary type, 50% cases in Metaplastic type, however single case (100%) of Mucinous type showed to be E-cad positive.

E-CADHERIN STATUS IN RELATION TO TUMOUR STAGE:

TABLE (18): E-Cad status in relation to tumour stage (n= 76)

E-Cad expression (In terms of membrane staining)	**Tumour staging**					**Statistics**
	T1	T2	T3	T4	Total	Chi-square value= **6.498,** Dof=**3,** p-value= **0.089**
Negative (0/1+)	5	13	17	0	35	
Positive (2+/3+)	8	23	9	1	41	
Total	13	36	26	1	76	

Shows E-Cadherin status in different tumour stages. E-Cad was found to be positive in the only T4 tumour in this study. Among 36 T2 tumours, E-Cad was Negative in 36% cases. In 26 T3 tumours, E-Cad Negativity was found in 65% cases. Lastly among 13 T1 tumours, E-Cad was Negative in 38% cases.

DISTRIBUTION OF TUMOR STAGES IN E-CAD NEGATIVE CASES

TABLE (19) : Distribution of tumour stages in E-Cad Negative cases (n= %)

Tumour Stage	**Frequency**	**Percentage %**
T1	5	14
T2	13	37
T3	17	49
T4	0	0
Total	35	100

Shows distribution of various tumour stages in E-Cadherin Negative cases. Among 35 E-Cad Negative cases T3, T2, T1 tumours were distributed in 49%, 37%, 14% respectively. No T4 tumour was found to be E-Cad Negative.

E-CADHERIN STATUS IN RELATION TO LYMPHNODE METASTASIS:

TABLE (20): E-Cad status in relation to Lymph Node metastasis (n= 76)

E-Cad expression (In terms of membrane staining)	Lymph Node status					Statistics
	N0	N1	N2	N3	Total	Chi-square value= **33.23,**
Negative (0/1+)	5	13	14	3	35	

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

Positive (2+/3+)	32	7	2	0	41	Dof=3**,** p-value= **0.000004**
Total	37	20	16	3	76	

Shows E-Cadherin status in tumours according to their nodal spread. E-Cad was Negative in 14% of N0 tumours, 65% of N1 tumours, 87% of N2 tumours and 100% of the N3 tumours. Taking 95% CI, plotting this into 4x2 table and applying chi-square test, it was found that loss of E-Cadherin expression had got highly significant association with lymph node metastasis (p- value < 0.05).

DISTRIBUTION OF LYMPH NODE METASTASIS IN E-CAD NEGATIVE CASES

TABLE (21): Distribution of lymph node metastasis in E-Cad Negative cases (n= %)

Lymph Node Stage	Frequency	Percentage %
N0	5	14
N1	13	37
N2	14	40
N3	3	9
Total	35	100

Shows distribution of 35 E-Cad Negative cases according to their lymph node status. N2 tumours (40%) were most common, whereas N1, N0 and N3 tumours were 37%, 14% and 9% respectively.

E-CADHERIN STATUS IN RELATION TO HISTOLOGIC GRADES:

TABLE (22): E-Cad status in different histologic grades (n= 76)

E-Cad expression (In terms of membrane staining)	Histologic Grade				Statistics
	Grade-I	**Grade-II**	**Grade-III**	**Total**	Chi-square value = **8.699,** Dof=**2,** p-value= **0.013**
Negative (0/1+)	1	9	25	35	
Positive (2+/3+)	6	19	16	41	
Total	7	28	41	76	

Shows E-Cadherin status in different histological grades of the tumours. E-Cad was Negative in 15% of Grade I tumours. In Grade II tumours 32% were E-Cad Negative and 61% of Grade III tumours were E-Cad Negative. This was found to be statistically significant (p-value ≤ 0.05).

DISTRIBUTION OF HISTOLOGICAL GRADES IN E-CAD NEGATIVE CASES

TABLE (23) : Distribution of histological grades in E-Cad Negative cases (n= %)

Histological Grade	Frequency	Percentage %
Grade 1	1	3
Grade 2	9	26

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

Grade 3	25	71
Total	35	100

Shows distribution of various histological grades in E-Cad Negative cases. 71% of Grade III and 26% of Grade II tumours constituted bulk of the 35 E-Cad Negative cases. Only 3% of total E-Cad negative cases were Grade I tumours.

E-CADHERIN STATUS IN RELATION TO MOLECULAR SUBTYPES:

TABLE (24): E-Cad status in different molecular subtypes (n= 76)

E-Cad expression (In terms of membrane staining)	Molecular subtypes					Statistics
	Luminal A	**Basal Like**	**Luminal B**	**Her2 enriched**	**Total**	Chi-square value = **14.023** Dof=**3,** p-value= **0.0029**
Negative (0/1+)	10	13	8	4	**35**	
Positive (2+/3+)	28	8	5	0	**41**	
Total	**38**	**21**	**13**	**4**	**76**	

Shows E-Cadherin status in different molecular subtypes. All the 4 HER2 enriched tumours were E-Cad Negative. 62% of both Basal like and Luminal B tumours were E-Cad Negative. 26% of Luminal-A tumours were E-Cad Negative. Hence, it was found that E-Cad expression had got significant association with molecular subtypes. (p-value ≤ 0.05)

DISTRIBUTION OF MOLECULAR SUBTYPES IN E-CAD NEGATIVE CASES

TABLE (25): Distribution of molecular subtypes in E-Cad Negative cases (n= %)

Molecular Subtype	**Frequency**	**Percentage %**
Luminal A	10	29
Luminal B	8	23
HER2/neu enriched	4	11
Basal Like	13	37
TOTAL	35	100

Shows distribution of different molecular subtypes in E-Cad Negative cases. Basal like molecular subtype was most common with 37%, followed by Luminal A and Luminal B molecular sub type with 29% and 23% respectively. Only 11% of the total E-Cadherin Negative tumours were Her2 enriched subtype.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

DISCUSSION:

Breast cancers are a diverse group of diseases that vary remarkably in terms of clinical presentation, histology, behaviour, and genetic characteristics. Since the 1930s, there has been a steady increase in the incidence of breast cancers worldwide. This increase continued steadily into the early nineties. It contributes to 15.3% of all female malignancies worldwide (SEER COMMITTEE) **[20]**.

Globalization is now titling this balance; adaptation of a western lifestyle and improved access to diagnostic modalities has been implicated in the increased rates in Asia, including India. Two established reasons have been attributed to high rates of incidence i.e., Increase in awareness and mammographic screening and use of hormone replacement therapy in post-menopausal women. Hence, it continues to be a major cause of morbidity and mortality throughout the world.

Several parameters have been investigated to predict the aggressiveness of breast cancer, such as lymph node status, tumour size, histologic type, tumour grade, hormonal receptor status, ploidy, and proliferating markers. The major prognostic factors include the invasive carcinoma versus in situ carcinoma, distant metastasis, lymph node metastases, tumour size, locally advanced disease, and inflammatory carcinoma.

Minor prognostic factors include histologic subtype, tumour grade, ER & PR receptors, Her2/neu expression, proliferative activity, and DNA content. Among these, E-Cadherin is one of the recently employed biological markers of tumour aggressiveness. Studies showed that loss of E-Cadherin expression are associated with aggressive tumour behaviour **[21-23]**. The association between loss or down regulation of E-Cadherin and the progression of sporadic breast cancer has been extensively documented. Both irreversible and reversible mechanisms are at play and the prevalence of each is related to the histologic subtypes.

The present study was conducted in the Department of Pathology at **M.K.C.G. MEDICAL COLLEGE AND HOSPITAL, BERHAMPUR** from the period of September 2018 to August 2020 taking 76 breast cancer cases. The age of presentation in this study ranges from 26 – 81 years with a mean age of 50 years. Majority of patients were present in the age group of ≥ 40 years (80%), although pre-menopausal age group patients (55%) were more commonly encountered. It is a documented fact that, advancement of age increases the risk of breast cancer, although there is evidence that Indian women are more likely to develop breast cancer at earlier ages than their Western counterparts **[24]**.

In the present study, out of total cases (i.e., n=76) majority (87%) were IDC NOS type followed by Lobular (5%), Medullary (4%), Metaplastic (3%), Mucinous (1%). Tumour size is one of the most powerful prognostic markers in breast cancer. In node-negative breast cancer cases, the single most important prognostic factor is tumour size and one of the strongest predictors for dissemination & rate of relapse in these cases. However, axillary node status is the single most important prognostic factor for patients with early breast cancer. Many studies had shown that treatment outcome is very poor in cases which had axillary lymph node metastasis as compared to node negative breast cancer cases.

In the present study maximum number of tumours (48%) were of size > 2 cm to ≤ 5 cm (T2 stage) which was similar to findings of C Suciu et al (41%) [25], Paul J Kowalski et al (43.3%) [26] and Layla et al (48.1%) [27]. but more than that of Qureshi et al (33.9%) [17] who found T1 tumours (56.6%) were most common. Lymph node metastasis seen in 51% of cases in this study which was more than the study of

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

Qureshi **et al (40.2%)** but much lower than other studies i.e., Layla et al (70.3%), Paul J Kowalski et al (72%).

Tumour grade is the description of a tumour based on how abnormal the tumour cells and tumour tissue look under a microscope and indicates how quickly the tumour is likely to grow and spread. It is the reflection of degree of malignancy in the morphology. Present study showed maximum cases were of grade III tumours (54%) followed by Grade II tumours (37%) and Grade I tumours (9%). However, **Kowalski et al (57%), Quereshi et al (41.7%)** and **C Suciu et al (47.4%)** found Grade II tumour as the most prevalent grade.

From the therapeutic point of view, breast cancer is divided into molecular/intrinsic subtypes based on hormone receptors (ER, PR) and HER2/neu status. Accordingly, these are of 4 types: Luminal A, Luminal B, HER2/neu enriched and Basal like (Triple negative). All these have targeted therapies except triple negative breast cancer (TNBC) which has only got chemotherapy as its mainstay of treatment and therefore it has the worst prognosis among all. It is documented that Luminal A is the most common and Basal like (TNBC) is the least common intrinsic subtype (10-15% of all breast cancers).

In this present study it was found that Luminal A (50%) was the most common type, followed by Basal like (28%), Luminal B (17%) and Her2/neu (5%) being the least common type, which corresponds to the study conducted by **Fulga et al** [28] but **Yang et al** [29] found Luminal B to be the most common type followed by Basal like. Immunohistochemical evaluation of E-Cadherin had been done to study its correlation with different tumour variables.

Correlation of E-Cadherin expression with different tumour variables:

Age: There was no significant association between E-Cad status and different age groups in the present study. Irrespective of the age of breast cancer patients, E-Cad was expressive in more than half of the cases.

Menstrual status: In the present study, it was found that Loss of E-Cad expression was significantly associated with menstrual status (p-value = 0.002) and was found to be 64% in pre and 30% in post-menopausal patients.

Histologic Type: The analysis of E-Cad distribution by histologic subtypes showed that 37 (56%) cases of IDC-NOS expressed this marker. E-Cad was positive in 2(66%) cases of medullary, one (50%) case of metaplastic and one (100%) case of mucinous carcinoma. The staining was strong linear at the cell borders of well and moderately differentiated tumours but was heterogeneous and dotted over cell borders in the high-grade tumours. All the lobular carcinoma cases were negative for E-Cad expression.

The results found were close to those of **Md Isa Nurismah et al** (56.3% preserved E-Cad expression) [30], **Gamallo et al** (50% of E-Cad positivity in IDC cases) [31], **Paul J Kowalski et al** (55% normal positivity) but less than those of **Hina S Qureshi et al** (99.5% positivity), **Rajeev Singhai et al** (99.5%) [32], **Kanthilatha Pai et al** (85.7% cases) [33] and **Layla et al** (72% positivity).
On the other hand, **C Suciu et al** found 45.5% cases with normal expression which was lesser than our study.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

In this present study, the expression of E-Cadherin in IDC and ILC was analysed to study its utility as a bio marker to help distinguish between the two tumour types. The result showed complete loss of E-Cadherin immunoreactivity in all four (100%) ILCs which was well documented in most of the literatures (**Gamallo et al, C Parker et al [34], Quereshi et al, Md Isa Nurismah et al, Rajeev Singhai et al, Kanthilatha Pai et al)**.

Most studies, including present reported that IDCs were mainly E-Cad positive, but expression was on occasions reduced. The loss of expression in lobular carcinomas is likely to be reflected in its distinct growth and invasion pattern. Lobular cancers tend to grow infiltratively, as opposed to the expansive pattern seen in many other cancers. Loss of E-Cad could lead to 'looser' intercellular connections, so cells can dissociate from each other and invade in strands or clumps, resulting in the characteristic 'Indian file' morphology seen histologically [34].

In the present study the maximum number of cases were IDC (NOS) type and very few numbers of other histopathological types; hence we could not find any significant association of E-Cad expression with histological type of tumour.

Tumour size (T stage): Present study did not show any significant correlation between loss of E-Cad expression with respect to tumour size. Most of the other studies also had similar findings. However, in the study done by **C Suciu et al,** it was seen that tumours over 2 cm in their larger diameter showed a decreased expression of E-Cadherin.

Lymph node status: With higher nodal metastasis E-Cad activity decreases and maximum number of E-Cad negative cases were seen in N3 stage followed by N2 and N1 stages. Node negative cases showed strong E-Cad expression (86%) and we had got a highly significant association between E-Cad and lymph node status (p-value = 0.000004) similar to the study of **Layla et al, Fulga et al, Yang et al**.

Histologic Grade: In the present study, an inverse relationship of E-Cad with histologic grade with high statistical significance (p-value = 0.013) was observed. 15% of the Grade I tumours, 32% of Grade II tumours and 61% of Grade III tumours were E-Cad Negative respectively. This corresponds to the results of **Gamallo et al**, **C Parker et al** and **C Suciu et al.** However, **Md Isa Nurismah et al** found maximum loss of E-Cad expression in Grade III tumours (71% cases) which was higher than our study results.

Molecular Subtype: In the present study, Luminal A phenotype showed highest expression of E-Cadherin (78%) followed by Luminal B and Basal like (38% each). All cases of Her2/neu were E-Cad negative in this study, showing significant association between loss of E-Cadherin and molecular subtype (p-value = 0.0029). The study conducted by **Yang et al** also showed similar findings and they postulated that ER-positive expression may be involved in the regulation of E-cadherin expression.

CONCLUSION:

The present study entitled **"EVALUATION OF E-CADHERIN EXPRESSION IN INVASIVE BREAST CARCINOMA AND ITS HISTO-PATHOLOGICAL CORRELATION"** was undertaken in department of Pathology, MKCG Medical college and Hospital, Berhampur, from the period of September 2018 to August 2020.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

- Total 76 cases of breast carcinoma were studied.
- The age of the patients ranged from 26 to 81 years with mean age of 50 years.
- Maximum patients were above 40 years of age and were pre-menopausal (80% and 55%, respectively).
- IDC NOS was the major histologic type constituting 87% of total. Other types found were Lobular (5%), Medullary (4%), Metaplastic (3%), and Mucinous (1%) variants.
- 36 out of 76 cases (48%) were of size > 2 cm to ≤ 5 cm (T2 stage).
- Lymph node metastasis was present in 39 cases and maximum were in N1 stage (26%).
- Histologic Grade III was the most common (54%) followed by Grade II (37%) and Grade I (9%).
- Most common molecular subtype was Luminal A (50%) followed by Basal Like/Triple Negative (28%), Luminal B (17%) and HER2 enriched (5%).
- E-Cadherin was found to be negative in 35 out of 76 cases (46%).
- Higher histologic grade and lymph node stage had decreased E-Cad activity.
- E-Cad expression was completely lost in Her2/neu enriched (100%) while maximally lost in Basal like/triple negative and Luminal B phenotypes (62% in each type).
- Loss of E-Cadherin expression in breast cancer correlates with Menstrual status, higher histologic grade, positive lymph node status and specific molecular subtypes (TNBC and Her2 enriched). In the present study we specifically determined the expression of E-Cadherin protein in primary invasive breast carcinomas and their histopathological correlation. Diminished E-Cadherin Expression in higher grades and positive lymph node cases supports the view that loss of E-Cadherin expression is a marker of aggressiveness.

Further, E-Cadherin can be a useful bio marker to differentiate between IDC and ILC since all Lobular carcinomas were negative for E-Cadherin expression. However, since there were only four cases of ILC, large scale studies are needed to establish this difference and also to confirm the prognostic value of E-Cadherin.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

Fig F1: ER, PR & HER2/neu STAINING

Fig F1(a) : ER +ve X 400 showing strong nuclear staining in > 70% of cells (score = 8)

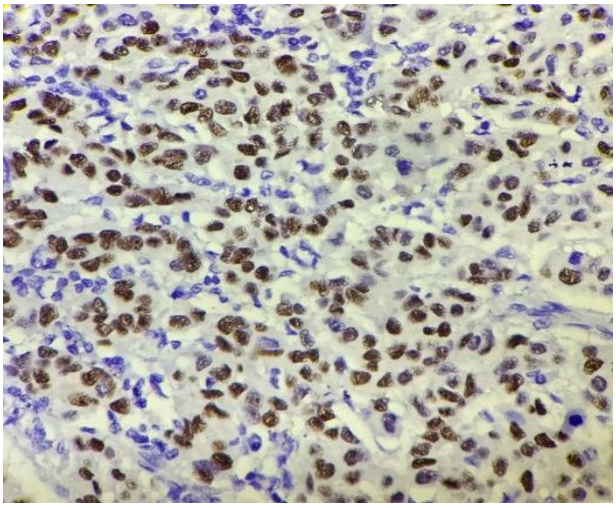


Fig F1(b) : PR +ve X 400 showing strong nuclear staining in > 50% of cells (score = 7)

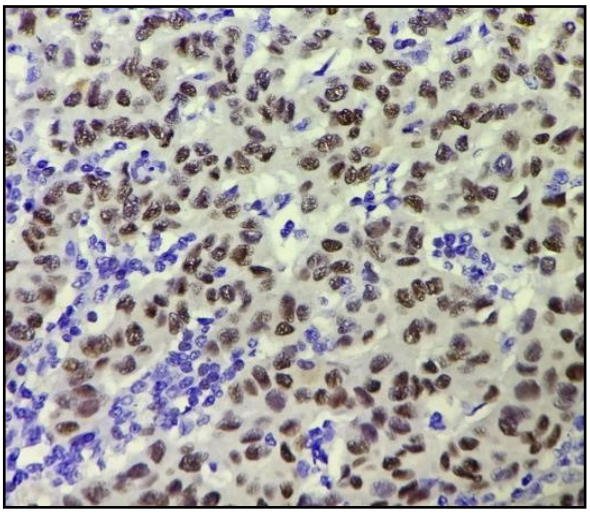


Fig F1(c): HER2/neu Equivocal X 400 showing weak complete membrane staining in > 10% of cells (score = 2+)

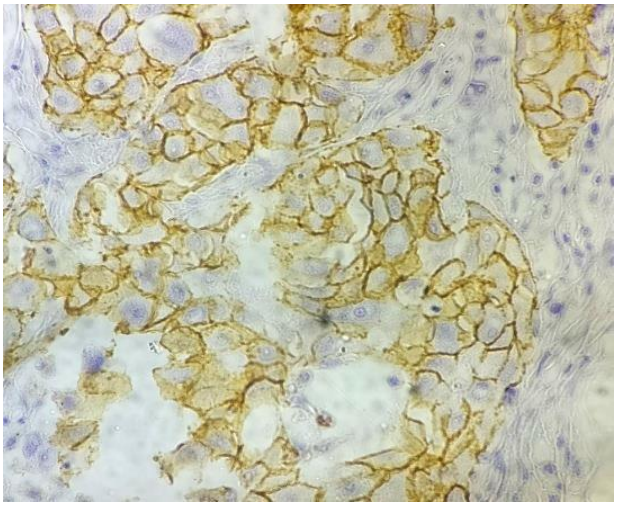
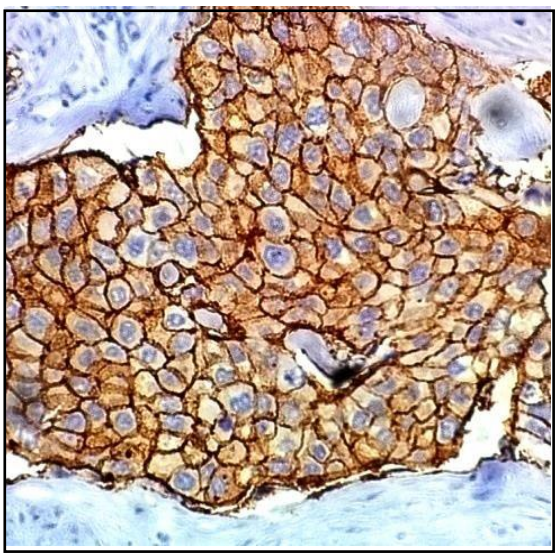


Fig F1(d) : HER2/neu Positive X 400 showing strong complete membrane staining in > 10% of cells (score = 3+)



ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

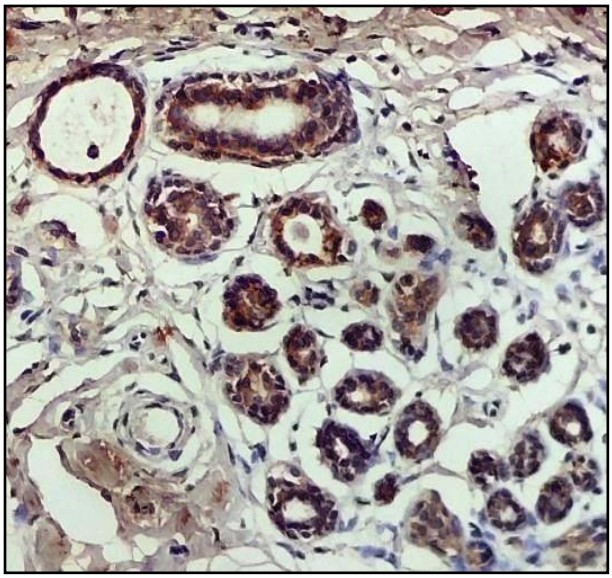


Fig F2(a) : E-CD +ve X 100 showing membrane staining in lobular and ductal epithelial cells of normal breast tissue

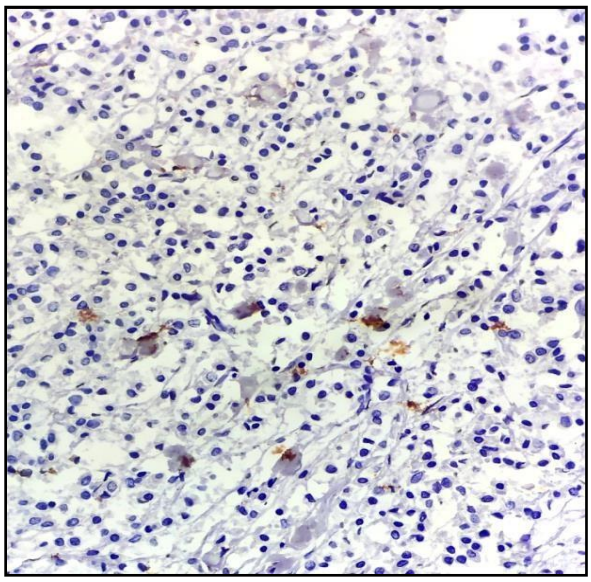


Fig F2(b) : E-CD -ve X 400 showing no membrane staining (score=0/1+)

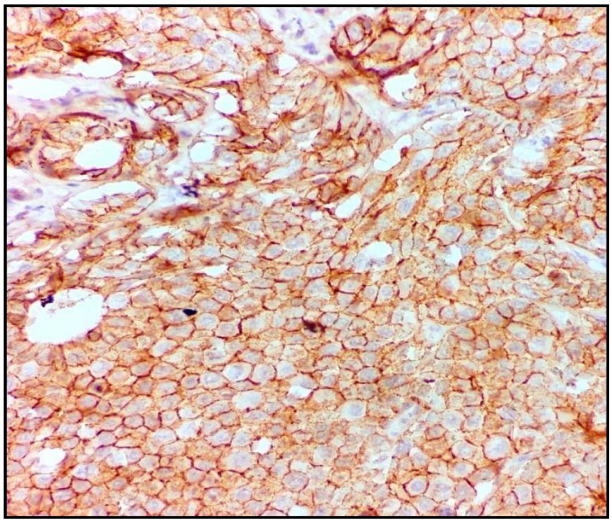


Fig F2(c) : E-CD +ve X 400 showing weak/ moderate complete membrane staining in > 10% of tumour cells (score=2+)

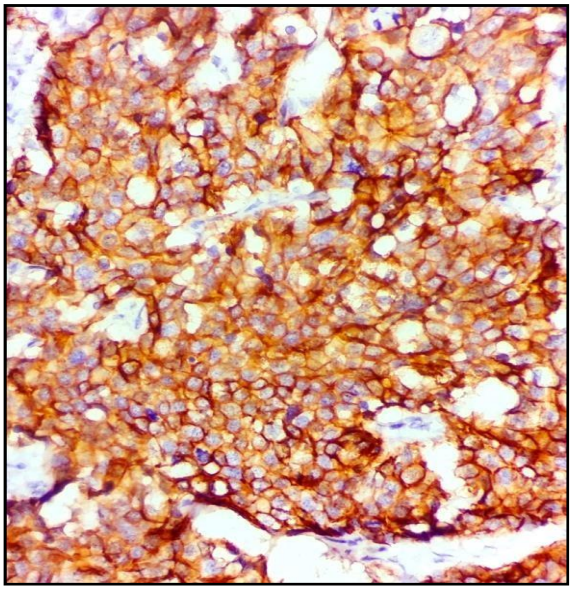


Fig F2(b) : E-CD +ve X 400 showing strong complete membrane staining in > 10% of tumour cells (score=3+)

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

BIBLIOGRAPHY

1) U.S Cancer Statistics Working Group. United States Cancer Statistics: 1999- 2014 Incidence and Mortality Web-based Report. Atlanta (GA): Department of Health and Human Services, Centres for Disease Control and Prevention, National Cancer Institute 2017.
Availaible at: http://www.cdc.gov/uscs
2) Ferlay J, Colombet M, Soerjomataram I, et al. Global and Regional Estimates of the Incidence and Mortality for 38 Cancers: GLOBOCAN 2018. Lyon: International Agency for Research on Cancer/World Health Organization; 2018.
3) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet- Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015; 65:87-108.
4) Berx G,Staes K, Van Hengel J,et al, Cloning and characterization of the human invasion suppressor gene E-Cathedein (CDH1) Genomics. 1995; 26:281289. [PubMed]
5) Bracke ME, Van Roy FM, Mareel MM. The E-Cathederin/catenin complex in invasion and metastasis. Curr Top Microbiol Immunol. 1996;213 9 (Pt 1): 123-161. [PubMed]
6) Frixen UH, Behrens J, Sachs M, et al. E-Cathederin medicated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol. 1991; 113:173-185. [PubMed]
7) Takeichi M.Cadherins in Cancer: implications for invasion and metastasis. Curr Opin Cell Biol. 1993; 5:806-811. [PubMed]
8) Tang A, Amagai M, Granger LG, Stanley JR, Udey MC. Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-Cadherin. Nature. 1993;361: 82-85. [PubMed]
9) Handschuh G, Candidus S, Luber B, et al. Tumor associated E-Cadherin mutations after cellular morphology, decrease cellular adhesion and increase cellular motility. Oncogene. 1999; 18:4301-4312. [PubMed]
10) Berx G, Cleton-Jansen AM, Nollet F, et al. E-Cadherin is a tumor/invasion suppressor gene mutated in human lobular breast cancers. EMBO J.1995; 14:6107-6115. [PubMed]
11) Vos CB, Cleton-Jansen AM, Berx G, et al. E-Cadherin inactivation in lobular carcinoma in situ of the breast: an early event in tumorigenesis. BR J Cancer. 1997; 76:1131-1133. [PubMed]
12) Kanai Y, Oda T, Tsuda H, Ochiai A, Hirohashi S. Point mutation of E-Cadherin gene in invasive lobular carcinomaof the breast. Jpn J Cancer Res. 1994;85: 1035-1039. [PubMed]
13) Elston CW. The assessment of histological differentiation in breast cancer. Aust N Z J Surg [Internet]. 1984 Feb [cited 2017 Nov 7];54(1):11–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6586161
14) 2019 WHO Classification of Tumors of the Breast (5th edition, Lakhani et al)
15) Bancroft's Theory and Practice of Histological Techniques, 7th Edition
16) Dako, Immunohistochemical Staining Methods, 6th Edition
17) Qureshi HS, Linden MD, Divine G, Raju UB. E-cadherin status in breast cancer correlates with histologic type but does not correlate with established prognostic parameters. Am J Clin Pathol. 2006 Mar;125(3):377-85. PMID: 16613340.
18) Mudduwa LKB. Quick score of hormone receptor status of breast carcinoma: correlation with the other clinicopathological prognostic parameters. Indian J Pathol Microbiol [Internet]. 2009 [cited 2017 Nov 7];52(2):159–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19332901
19) HER2 Scoring Criteria Update for Breast Cancer AUTOMATED BREAST CANCER

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 01, 2023

HER2/NEU FISH ANALYSIS Test Description Interpretation and Results Photomicrograph. 2013 [cited 2017 Nov 7]; Available from: https://www.storm-path.com/Files/91643 - HER2 Guidelines.pdf

20) Kumar, Abbas, Faustor, Aster. *Robbins and Cotran Pathologic Basis of Disease*, 10th ed. Philadelphia PA: Elsevier-Saunders; 2020.

21) Moll R, Mitze M, Birchmeier W. Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. American journal of pathology (1993)143,1731- 42.

22) Berx G, Cleton-Jansen AM, Nollet F, de Leeuw WJ, van de Vijver M, Cornelisse C, van Roy F: E-cadherin is a tumour/invasion suppressor gene mutated in human lobular breast cancers. EMBO J 1995, 14:6107-6115.

23) Howard E M, Lau AK, et al. (2005). Expression of E-cadherin in high-risk breast cancer. J cancer res Clin Oncol (2005) 131: 14-18.

24) Statistics of Breast Cancer in India: Trends in India [Internet]. [cited 2017 Nov 17]. Available from: http://www.breastcancerindia.net/statistics/trends.html

25) Suciu C, Cîmpean AM, Mureşan AM, Izvernariu D, Raica M. E-cadherin expression in invasive breast cancer. Rom J Morphol Embryol. 2008;49(4):517-23. PMID: 19050801.

26) Kowalski PJ, Rubin MA, Kleer CG. E-cadherin expression in primary carcinomas of the breast and its distant metastases. Breast Cancer Res. 2003;5(6): R217-22. doi: 10.1186/bcr651. Epub 2003 Sep 26. PMID: 14580257; PMCID: PMC314411.

27) Younis LK, El Sakka H, Haque I. The Prognostic Value of E-cadherin Expression in Breast Cancer. Int J Health Sci (Qassim). 2007 Jan;1(1):43-51. PMID: 21475451; PMCID: PMC3068666.

28) Fulga V, Rudico L, Balica AR, Cimpean AM, Saptefrati L, Margan MM, Raica M. Differential expression of e-cadherin in primary breast cancer and corresponding lymph node metastases. Anticancer Res. 2015 Feb;35(2):759-65. PMID: 25667455.

29) Yang L, Wang XW, Zhu LP, Wang HL, Wang B, Zhao Q, Wang XY. Significance and prognosis of epithelial-cadherin expression in invasive breast carcinoma. Oncol Lett. 2018 Aug;16(2):1659-1665. doi: 10.3892/ol.2018.8836. Epub 2018 May 30. PMID: 30008850; PMCID: PMC6036376.

30) Nurismah MI, Noriah O, Suryati MY, Sharifah NA. E-cadherin expression correlates with histologic type but not tumour grade in invasive breast cancer. Asian Pac J Cancer Prev. 2008 Oct-Dec;9(4):699-702. PMID: 19256762.

31) Gamallo C, Palacios J, Suarez A, Pizarro A, Navarro P, Quintanilla M, Cano A. Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. Am J Pathol. 1993 Apr;142(4):987-93. PMID: 7682767; PMCID: PMC1886894.

32) Singhai R, Patil VW, Jaiswal SR, Patil SD, Tayade MB, Patil AV. E-Cadherin as a diagnostic biomarker in breast cancer. N Am J Med Sci. 2011 May;3(5):227-33. doi: 10.4297/najms.2011.3227. PMID: 22558599; PMCID: PMC3337742.

33) Pai K, Baliga P, Shrestha BL. E-cadherin expression: a diagnostic utility for differentiating breast carcinomas with ductal and lobular morphologies. J Clin Diagn Res. 2013 May;7(5):840-4. doi: 10.7860/JCDR/2013/5755.2954. Epub 2013 Mar 18. PMID: 23814724; PMCID: PMC3681051.

34) Parker C, Rampaul RS, Pinder SE, Bell JA, Wencyk PM, Blamey RW, Nicholson RI, Robertson JF. E-cadherin as a prognostic indicator in primary breast cancer. Br J Cancer. 2001 Dec 14;85(12):1958-63. doi: 10.1054/bjoc.2001.2178. PMID: 11747340; PMCID: PMC2364028.