

Original Research Article

Evaluation of Tumour ER/PR Status and HER2/Neu Expression by Immunohistochemistry (IHC) as a Prognostic and Predictive Factors in Breast Carcinoma

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Abstract

Background and Objectives: Breast carcinoma is one of the leading causes of malignancy in females. Assessment of ER/PR and HER2/neu in breast cancer is mandatory in clinical practice. IHC for assessing hormonal receptor status is easier, safer, and has better ability to predict response to adjuvant endocrine therapy. HER2/neu overexpression is shown to have important prognostic and predictive value. The best approach to the use of immunohistochemical markers is to couple them with standard H&E histology and to use panel of markers. The purpose of the study was to assess the ER, PR & HER2/neu status and correlate with clinicopathological parameters.

Methodology: 50 cases of breast carcinoma from July 2015 to June 2017 were taken for the study. H&E sections diagnosed as carcinoma were assessed for histological type and grade. Allred scoring system to score ER/PR status and ASCO/CAP guidelines for HER2/neu status were used. Histopathological grading was done according to Modified Scarf Bloom-Richardson's method.

Results: In the present study, the most common histological subtype was infiltrating ductal carcinoma (88%), and the most common immunohistochemical subtype was triple negative (40%). Most of the tumors were of histological grade II.

Interpretation & conclusion: ER, PR, & HER2/neu status correlates well with histopathological grading and other clinicopathological parameters. Hence, immunohistochemical analysis should be incorporate in the routine histopathology reports and can be of great value in deciding the treatment protocols.

Keywords: Breast Carcinoma; Immunohistochemistry; Er; Pr; Her-2/Neu; Triple Negative Breast Carcinoma.

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Introduction

Carcinoma of the breast remains one of the leading cancers in women today with an estimated life time risk of 13% [1].

Approximately three fourth of mammary carcinoma are invasive ductal type. Within these, and also among other morphologic categories, tumours

display marked heterogeneity in many of their biologic properties. One is the expression of steroid receptors in concern with the oncogene Human epidermal growth factor receptors 2 (HER2). This has important clinical implications, such as selection of patients for endocrine therapy [2,3].

Major Prognostic Factors are Invasive carcinoma versus in situ disease, distant metastases, lymph

node metastases, tumor size, locally advanced disease and inflammatory carcinoma and Minor Prognostic and Predictive Factors are histologic subtype: the 30-year survival rate of women with special types of invasive carcinomas (tubular, mucinous, medullary, lobular, and papillary) is greater than 60%, compared with less than 20% for women with NST cancers, histologic grade.; the most commonly used grading system, the Nottingham Histologic Score (also referred to as Scarff-Bloom Richardson), combines nuclear grade, tubule formation, and mitotic rate to classify invasive carcinomas into three groups that are highly correlated with survival, Estrogen and Progesterone receptors, HER2/neu, Lymphovascular invasion, Proliferative rate, DNA content, Response to neoadjuvant therapy and gene expression profiling Newer therapeutic strategies include inhibitors of membrane- bound growth factor receptors (e.g. HER-2/neu), stromal proteases and angiogenesis [4].

Presence or absence of tumour estrogen receptors (ER) or progesterone receptors (PR) directly related with ultimate clinical outcomes. We evaluate tumour ER/PR status and HER2/neu expression by Immunohistochemistry (IHC) as a prognostic and predictive factors in breast carcinoma. HER2/neu was one of the first oncogenes studied in invasive breast cancers [5]. Its earlier significance as a prognostic factor has been surpassed by its key importance as a biomarker for sensitivity to herceptin (trastuzumab, a monoclonal antibody against the HER2/neu receptor) and resistance to tamoxifen which aids targeted therapy [6].

Estrogens are primarily responsible for proliferation of mammary ducts and progesterone for the development of lobules [7].

Tumors of the breast is classified according to WHO 2012 classification [8]. It is overwhelmingly a disease of females (female to male ratio of approximately 200 to 1) [9]. In India, cancer of the breast is the most common cancer among women in many regions and has overtaken cervix cancer, which was the most frequent cancer a decade ago [10].

Histologic grading: Bloom chose to follow the Patey and Scarff method and seems to have paid more attention to mitotic figures than Patey and Scarff. Together with Richardson, Bloom is also responsible for the addition of a numerical scoring system [11]. Haagensen in 1933 evaluated 15 histologic features, the important being growth pattern, cell morphology and reaction of surrounding stroma [12].

The Patey and Scarff method, modified by Bloom and Richardson, subsequently showed correlation between grade and prognosis. The method was also adopted as the preferred grading system by the WHO [11]. Elston and Ellis published the Nottingham modification of the Bloom and Richardson method, which involves estimation of the percentage of tubule formation, the degree of nuclear pleomorphism, and an accurate mitotic count, using a defined field area [14] (Table 1).

Histological grading may be carried out in all cases of invasive mammary adenocarcinoma regardless of morphological type; tumours which are entirely of in situ type, or those in which the invasive component is minimal, are not suitable for grading. Medullary carcinoma is perhaps the only subtype for which this approach might not be appropriate. By definition these tumours are histological grade 3, but have been considered by some to have a more favorable prognosis than this degree of differentiation would imply [2]. In addition to pathological grade and stage breast cancers are routinely assessed for hormone receptor status (ER and PR) by IHC and human epidermal growth factor receptor 2 (HER2) expression by IHC. Patients with ER +ve primary tumours are offered adjuvant hormone therapy, routinely tamoxifen for 5 years, while post menopausal women may receive an aromatase inhibitor. Patients with overexpression of HER2 are eligible for trastuzumab, a mAb that targets the Her2/neu receptor [14].

Cancers with +ve ER findings respond favourably to hormone or endocrine therapy in

Table 1: Histologic Grading Using Nottingham Modification of Scarf Bloom Richardson System

Criteria	Score-1	Score-2	Score-3
1. Tubule formation	>75% of tumor	10-75% of tumor	<10% tumor.
2. Nuclear pleomorphism	Minimal variation in size and shape of nuclei	Moderate variation in size and shape of nuclei	Marked variation in size and shape of nuclei
3. Mitotic count per 10 hpf (0.44mm) filled diameter	0-5	6-10	>11

The three values are added to produce scores of 3 to 9 and the grading is one as follows:

Grades	Scores
Grade I	3-5
Grade II	6-7
Grade III	8-9

approximately 60% of patients and when both receptors are present, there is a 70-75% response rate. ER status does not predict the tumours response to chemotherapy [15]. Only 30-40% of trastuzumab patients benefit [13].

ER-positive cells, in the normal breast, do not show proliferative activity. In contrast, ER-positive cells in atypical ductal hyperplasia, and in invasive and in situ carcinoma, show proliferative activity. The change from an ER-positive non proliferating cell phenotype to an ER-positive proliferating cell phenotype appears to be a critical switch and is one of the characteristic events of malignancy. The exact mechanism that is responsible for this switch is as yet not understood, although deregulation of transforming growth factor beta signaling is believed to be involved [17].

Estrogen-receptor (ER)- positive tumours are heterogeneous, and the efficacy of hormonal therapy depends on status of multiple other proteins including other transcription factors such as FOXA1, GATA-3, growth factors, and co-activator and co-repressor proteins [17].

Allred score, which represented the estimated proportion of positive staining tumour cells (0-none; 1- Weak, 2-intermediate; and 3 -strong). The proportion and intensity scores are added to obtain a total score, which ranged from 0 to 8. Tumours were defined as hormone receptor negative if their score was 0 or 2 (Table 2) [18].

Currently, the only recommended predictive markers in oncology are ER and PR for selecting endocrine-sensitive breast cancers and HER-2 for identifying breast cancer patients with metastatic disease who may benefit from trastuzumab. For malignancies other than breast cancers, validated predictive markers do not exist at present [19].

ER/PR

Dunnwald KL et al. studied breast cancers and observed that the higher relative risks of mortality

Table 2: Allred scoring method for quantifying IHC results for ER/PR in breast cancers [8].

Proportion of positive cells	Score	Intensity of staining	Score	Total (Proportion score + Intensity score)
Nil	0	None	0	0
< 1%	1	Weak	1	2
1%-10%	2	Intermediate	2	3
11%-33%	3	Strong	3	4
34%-66%	4			5
67%-100%	5			6

associated with having an ER+/PR-, an ER-/PR- or an ER-/PR- tumor relative to an ER+/PR+ tumor were consistently present across all tumor characteristics. Compared to women with ER+/PR+ tumors, those with ER-/PR- tumors had increased risks of mortality across all histologic classification, suggesting that combined ER/PR negativity has implications for relative mortality risks, regardless of tumor histology. The one noted expression was that ER/PR- status did not appear to be related to the relative risk of mortality among women with medullary carcinomas. Although they are typically high grade their prognosis is more favorable than that of other invasive breast carcinomas [20].

Lakhani SR et al observed that Breast cancers in patients with *BRCA1* germline mutations are more often negative for estrogen receptor, progesterone receptor, and HER-2, and are more likely to be positive for p 53 protein compared with controls. In contrast, *BRCA2* tumors do not show a significant difference in the expression of any of these proteins compared with controls [21].

ER+/PR- and ER-/PR+ tumors are biologically and clinically distinct groups of breast cancer that may require different treatment strategies with ER- /PR + exhibiting more aggressive behavioral characteristics [22].

Fisher B et al. studied, 157 histologically node negative breast cancer patients of with ER/PR and with nuclear or histologic grade of tumors and correlated the Disease-Free Survival (DFS), Distant Disease-Free Survival (DDFS), and Survival (S). They found that combined with ER, PR made no independent contribution in the outcome prediction. Nuclear grade was the most important single marker of outcome [23].

Guera I et al. in 2003 proposed a new immunohistochemical prognostic index (IHPI) for breast cancer in women < 35 years. It was constructed as follows; one point was assigned for each positive value for either c-erbB-2 or p53 and zero points were given for negative values; one point was given for any negative value of ER or PR and zero points were given when positive. Tumors were then ranked from 0 to 4 points (the higher the index, the worse the prognosis), and grouped in good prognosis (0-1 points), moderate prognosis (2 points), or poor prognosis (3-4 points). This IHPI was useful in determining the prognosis of tumors ≤ 2cm and of moderate use for tumors >2-5cm and no use in tumors >5cm [24].

ER and PR is a reflection of differentiation and loss of this differentiated function i.e., ER and PR

negativity would reflect a more rapid growth rate. Therefore more rapidly growing tumors may be more sensitive to chemotherapy [25].

HER2

Berger MS et al found that there was a statistically significant correlation between c-erbB-2 protein expression and parameters used in breast cancer prognosis.

Positive staining was associated with positive nodal status of the patient ($P = 0.02$) and with tumors showing a poor nuclear grade ($P = 0.02$). This is the first study showing that a determination of the level of c-erbB-2 protein in paraffin-embedded tumor sections may have prognostic value for the course of human breast cancer [26].

Triple Negative Breast Cancer (TNBC)

Triple negative breast cancer, defined as that with negative expression of estrogen and progesterone receptors and HER-2 accounted for 10-17% of all breast carcinomas [27]. Regardless of stage at diagnosis, women with triple-negative breast cancers had poorer survival than those with other breast cancers, and non-Hispanic black women with late-stage triple negative cancer had the poorest survival, with a 5-year relative survival of only 14% [28].

Morphological Types in Carcinoma Breast

WHO Classification is based on the growth pattern and cytologic features and does not imply histogenesis or site of origin within mammary duct system. All carcinomas are thought to arise from terminal duct lobular unit. The most common histologic type of invasive breast cancer by far is invasive ductal carcinoma not otherwise specified [29].

Invasive ductal carcinoma – NOS type

Rosen (1975) accounts that this type constitutes 65-80% of mammary carcinomas. Microscopically, architectural arrangement may be in cords, clusters and trabeculae while some are characterized by predominantly solid or syncytial infiltrative pattern [30].

In a study conducted by Paul Peter Rosen et al., these carcinomas show 70%-80% ER/PR positivity [31]. According to Lakhmini KB Mudduwa the prevalence of hormone receptor positive breast cancer in Asian countries has found to be lower than western world where more than 50% tumors express hormone receptors [32].

Pleomorphic carcinoma is a rare variant of high grade ductal carcinoma- NOS characterized by pleomorphic and bizarre giant cells in more than 50% of tumor cells in a background of adenocarcinoma [28].

In a study conducted by Ellis IO et al, 10 yrs survival rate of this tumour ranges from 33-48% [33].

Invasive lobular carcinoma

In a study conducted by Grazio Arpino et al., this type represents 4.9-15% of all invasive breast carcinomas [34]. They are frequently bilateral and multicentric when compared with other subtypes [10].

Cristofanilli M et al. observed that patients with ILC tend to be older (median age, 53 years *v* 47 years for patients with IDC) and have more hormone receptor- positive tumors (92% *v* 62%; $p < .001$), lower nuclear grade (nuclear grade 3, 16% *v* 56%; $p < .001$), and higher stage at diagnosis (10% *v* 0% with stage IIIB or IIIC disease; $p < .001$) [35].

Tubular Carcinoma and mucinous carcinoma

Microscopically tubular carcinoma has irregularly arranged tubules lined by single layer of epithelial cells with little pleomorphism and low mitotic rate. The tubules are characteristically angulated and have open glandular lumina. It has two morphological types, pure type with stellate nature and sclerosing type with more diffuse ill defined nature. Pure tubular carcinoma has an excellent prognosis [36].

Microscopically, the mucinous carcinoma consists of small islands or clusters of epithelial cells floating in lakes of extracellular mucin divided by delicate fibrous septae. The lakes of mucin are positive for PAS and mucicarmine stain. These tumours carry a very good prognosis [8].

Diab GS et al observed that tubular and mucinous carcinomas were ER and PR positive, have a lower S-phase fraction, and epidermal growth factor receptor negative compared with NOS carcinomas. Axillary node involvement was a poor prognostic feature in mucinous but not tubular carcinomas. Mucinous carcinomas ≤ 1 cm had a $\leq 5\%$ incidence of node involvement [36].

Medullary carcinoma

Grossly the tumor appears as well circumscribed, soft and fleshy. WHO defines it as well circumscribed carcinoma composed of poorly differentiated cells with scant stroma and prominent lymphocytic

infiltration. They carry good prognosis with 10 year survival rate of 84 % [37].

They typically show basal-like biological features which are triple negative [54].

Papillary carcinoma

Papillary carcinoma is seen predominantly in postmenopausal patients.

Microscopically, it is circumscribed, show delicate or blunt papillae with focal solid areas of tumour growth. DCIS is present in > 75% of cases and usually has papillary pattern [34]. These lesions are characterized by indolent behavior and extremely favorable prognosis [38]. In a study conducted by Zekioglu et al hormone receptor positivity is seen in 89% of cases [39].

Metaplastic Carcinoma

Comprising less than 1% of invasive carcinomas of the breast, metaplastic carcinomas are group of malignant tumors in which part or all of the carcinomatous epithelium is transformed into a non glandular (metaplastic) growth process [40]. This heterogeneous group of tumors includes invasive carcinomas with squamous differentiation, or osseous or cartilaginous components, with mixed epithelial and spindle cell components of widely varying proportions, and keratin positive tumors with a pure sarcomatous appearance, metaplastic carcinomas are almost invariably negative for estrogen and progesterone receptors and for HER2/neu over expression [41]. It behaves as a highly malignant tumour with early recurrence and poor survival [42].

Neuroendocrine carcinoma

WHO defines this type as a carcinoma with neuroendocrine marker positivity noted in more than 50% of the cell population [8,43]. This type has as infiltrative morphology with component cells arranged in nests, sheets or trabecular formation and peripheral palisading of cell groups [44]. In a study conducted by Niremudi et al, 55-65 % showed ER, PR positivity [44].

Secretory carcinoma

According to the 2002 World Health Organization classification of breast tumors, secretory carcinomas are considered one of the rarest types of breast carcinomas, accounting for less than 0.15% of all breast cancers.

As the name implies, the characteristic histomorphology is the presence of a large amount

of intracellular and extracellular, eosinophilic secretion material that stains positive for diastase-resistant, periodic acid-Schiff. Most tumors stain positive for S100 and negative for estrogen receptor, progesterone receptor, and ERBB2 (formerly HER2/neu) (ie, triple negative). In addition, some secretory carcinomas demonstrate a basal-like immunoprofile [45].

Male Breast carcinoma

These are more likely to be high grade with retained expression of ER &PR. However, less likely to express HER2/neu [46].

Aims and Objectives

1. To study the ER, PR & HER-2/neu status in malignant breast lesions.
2. To correlate the ER, PR & HER-2/neu status with clinicopathological prognostic parameters.

Materials and Methods

A prospective study on "Evaluation of estrogen and progesterone receptors and HER-2/neu expression in malignant breast lesions" was conducted over a period of 2 years from July 2015 to June 2017, in the Department of Pathology, Khaja Banda Nawaz Institute Of Medical Sciences, Kalaburagi.

Sample size: 50 cases

Inclusion criteria

1. All epithelial malignant tumours of breast.

Exclusion criteria

1. All benign tumors of breast.
2. All the stromal tumours of breast .
3. Tumour like conditions.
4. Chemotherapy/radiotherapy treated patients.

The specimens were thoroughly examined and clinical details were analysed. All the cases were subjected to detail study

The specimen sent in formalin was sliced at 1 cm interval and fixed immediately in 10% NBF. One dedicated block from the tumor not fixed for more than 24 hours in formalin was used for IHC. Four μ thickness sections were cut and taken on Poly-L-Lysine coated slides and stained for evaluating ER, PR receptors and HER2/neu expression. And also sections were routinely stained

with H& E and histological grading of tumour was done on H&E stained sections according to Modified Bloom - Richardson grading and ER/PR nuclear staining was scored according to Allred score method and HER2 membrane staining was interpreted according to ASCO/CAP guidelines.

Results

In the present study (32%) were modified radical mastectomy (Figure 3) and 12 specimens (24%) were lumpectomy, out of 50 cases, the age of the patients ranged from 24 -90 years with mean age of 49.96 years. Majority, 16 cases (32%) belong to age group 41-50 years followed by 12 cases (24%) in age group 51-60 years. Out of 50 cases, most of the patients were premenopausal i.e 27 cases (54%) and rest were postmenopausal i.e 23 cases (46%).

In the present study, 29 cases (50%) presented on the left side where as 21cases (42%) presented on the right side.

Out of 50 cases, 25 cases (50%) showed tumor in the upper outer quadrant, followed by 11 cases (22%) in lower outer quadrant, 5 cases (10%) in upper inner quadrant, 5 cases (6%) in lower inner quadrant and 4 cases (8%) in subareolar region.

In the present study, the size of tumour measured between 2.0-5.0 cms in 36 cases (72%), followed by >5 cms in 10 cases (20%) and ≤2cms in 4 cases (8%).

In the present study, the predominant histologic subtype was infiltrating ductal carcinoma (NOS) accounting for 44 cases (88%), followed by 2 cases (4%) of mucinous carcinoma, 1 case (2%) of medullary carcinoma, 1 case (2%) of papillary carcinoma and 1 case (2%) of secretory carcinoma (Graph 1).

Necrosis was the most common histological finding in breast carcinoma cases seen in 26 cases (52%) out of which 2 cases (4%) having comedo necrosis.

Desmoplasia was seen in 23 cases (46%), lymphocytic infiltration 10 cases (20%), adipose tissue infiltration 8 cases (16%), lymphovascular invasion 15 cases (30%) and calcification in 4 cases (8%). Adjacent tissue show fibrocystic (10%).change in 5 cases (Table 3)

In the present study, 26 cases (52%) had score-3, 19 cases (38%) had score-2 and 5 (10%) had score-1 for tubule formation. 23 cases (46%) had score-3, followed by 22 cases (44%) had score-2 and 5 cases (10%) had score-1 nuclear grade. 19 cases (38%) had score-3, followed by 18 cases (36%) had score-2 and 13 cases (26%) had score-1 mitotic rate (Table 4).

In our study, the histological grading shows majority of cases in Grade II, 26 cases (52%) followed by 18 cases (36%) in Grade III and 6 cases (12%) in Grade I (Graph 2).

Out of 50 cases, 20 cases (40%) had nodal metastasis, 4 cases (8%) were negative for tumor deposits and in 26 cases (52%) nodes were not available for examination.

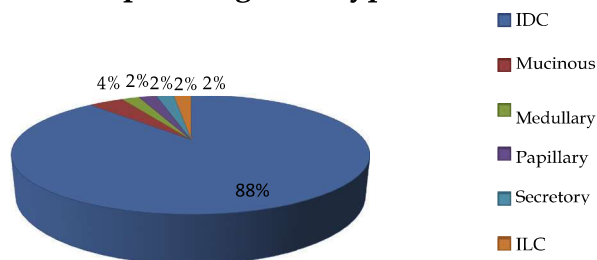
Immunohistochemical results

In the present study majority cases were Her2/neu negative (32 cases,64%) and ER/PR negative (30 cases, 60%). ER/PR positive were (20 cases,40%)

Table 3: Distribution of breast carcinoma cases according to associated histologic features

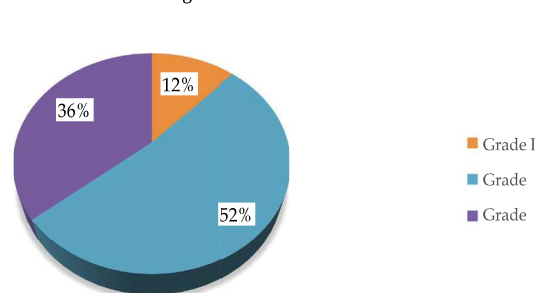
Histologic features	No.of cases	%
Necrosis	26	52
• Comedo	02	04
Desmoplasia	23	46
Calcification	04	08
Adipose Tissue Infiltration	08	16
Lymphocytic Infiltration	10	20
Lymphovascular invasion	15	30
Fibrocystic change	05	10

Histopathologic subtype



Graph 1: Distribution of breast carcinoma cases according to histologic subtype

Histological Grade



Graph 2: Distribution of Breast Carcinoma Cases According To Histologic Grade (Nottingham Modification of the Bloom Richardson System)

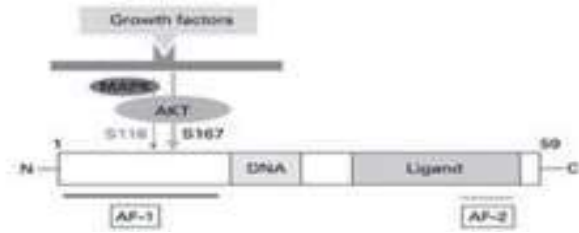


Fig. 1: Structure of the estrogen receptor(ER) α gene is shown With its DNA- binding domain and activating function (AF)-1 and AF-2 domains. Also note that the ER can be activated even the absence of estrogen by growth factor pathways by phosphorylation of serine residues S118 and S167 by MAPK and AKT, respectively.



Fig. 2: Structure of ER and PR: ER consists of two isoforms, (ER α & ER β) that are transcribed from two genes. PR also consists of two isoforms (PR-A & PR-B) that are transcribed from a single gene using an alternative promoter 7 transaction start site. DBD-DNA binding domain; LBD-Ligand Binding domain.



Fig. 3: Gross Picture: Left mastectomy specimen, cut section shows grey white mass infiltrating into adjacent tissue.

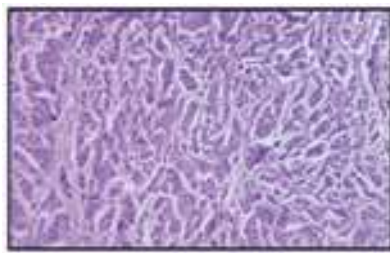


Fig. 4: Infiltrating Ductal Carcinoma (NOS) showing cells arranged in nests [H&E 100x]

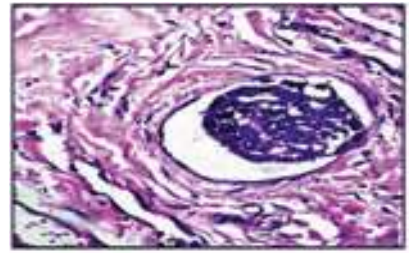


Fig. 5: Lymphovascular emboli [H&E 400x]

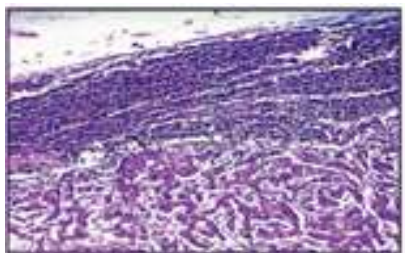


Fig. 6: Lymph node metastasis from IDC [H&E 100x]

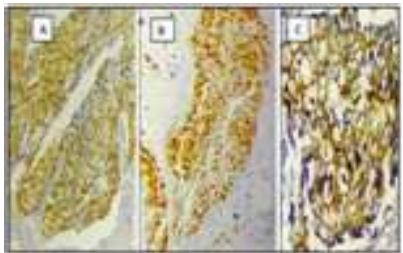


Fig.7: Infiltrating Ductal Carcinoma (NOS) showing A) ER/ (+ve), B) PR(+ve) and C) HER2/ neu(+ve).

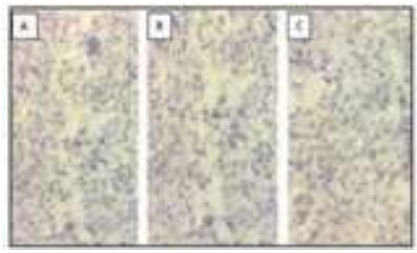


Fig. 8: Infiltrating Ductal Carcinoma (NOS) showing A) ER(-ve) B) PR(-ve) and C) HER2/ neu(-ve).

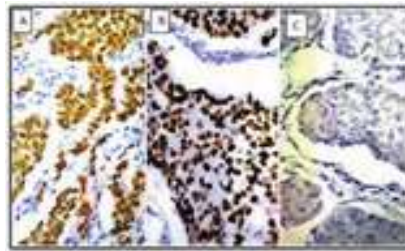


Fig. 9: Infiltrating Ductal Carcinoma (NOS) showing A) ER (+ve) B) PR (+ve) and C) HER2/ neu(-ve).

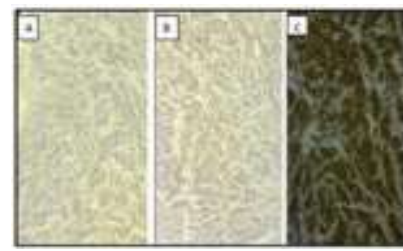


Fig. 10: Infiltrating Ductal Carcinoma (NOS) showing A) ER (-ve) B) PR (-ve) and C) HER2/ neu(+ve).

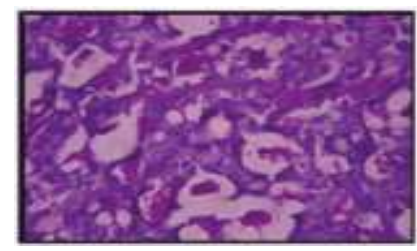


Fig. 11: Secretory carcinoma -'PAS' shows a diastase resistant positive reaction for secretory material.

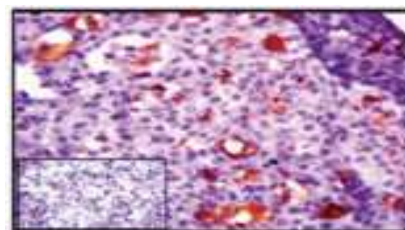


Fig. 12: Secretory carcinoma with ER (-ve) (secretions showed false +ve staining, Inset-PR(-ve))



Fig. 13: Medullary carcinoma : Anastomosing sheets of cells [H&E 100x] Inset- Cells with grade 3 morphology & lymphoplasmacytic infiltration [H&E 400x]

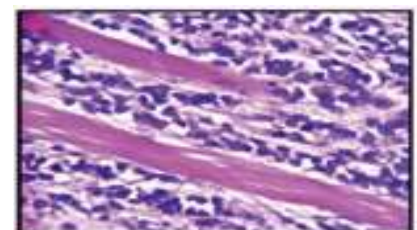


Fig. 14 (b): Invasive lobular carcinoma - Tumour cells are small, uniform, round having round to oval vesicular nucleus with nucleoli in some cells and scanty cytoplasm

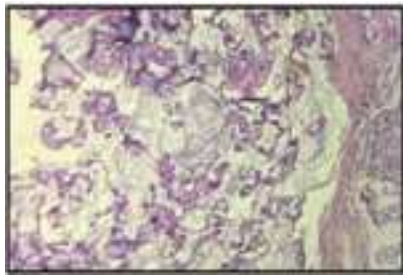


Fig. 15: Mucinous carcinoma with pools of mucin in between

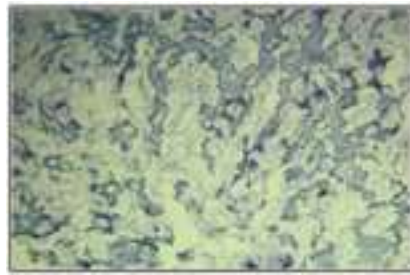


Fig. 16: Mucinous carcinoma showing PR (-ve)

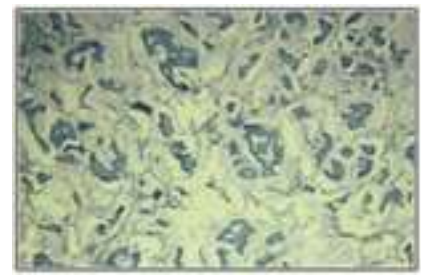


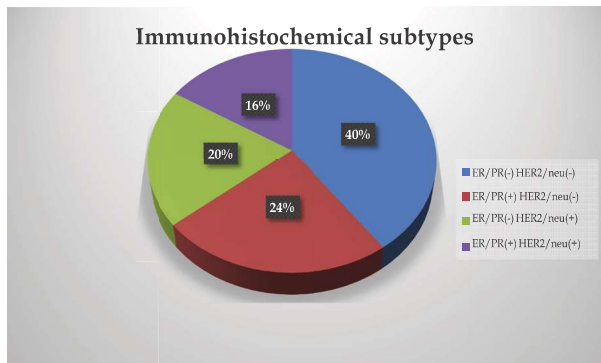
Fig.17: Mucinous carcinoma showing HER2/neu(-ve)

and Her2/neu positive were (18 cases, 32%)

Out of 50 cases, 20 cases (40%) were triple negative, 12 cases (24%) were ER/PR (+) HER2/neu(-), 10 cases (20%) were ER/PR(-) HER2/neu(+) and 8 cases (16%) were triple positive (Graph 3).

Out of 50 cases, 20 cases were triple negative, among them maximum cases were seen in the age group 41-50years (7 cases, 35%) and minimum cases were seen between 71-80years (1 case,5%).

Among 12 cases of ER/PR(+) HER2/neu(-) maximum cases were seen in age group 31-40years (4cases, 33.34%). Of the 10 cases of ER/PR(-) HER2/neu(+) maximum cases were seen in between 41-50years (5 cases, 50%). Among the triple positive cases (16%) maximum cases seen between 41-50 years (3 cases, 37.5%) and minimum in age group 61-70years (1 case,12.5%)



Graph 3: Distribution of Breast Carcinoma Cases According to Immunohistochemical Subtypes

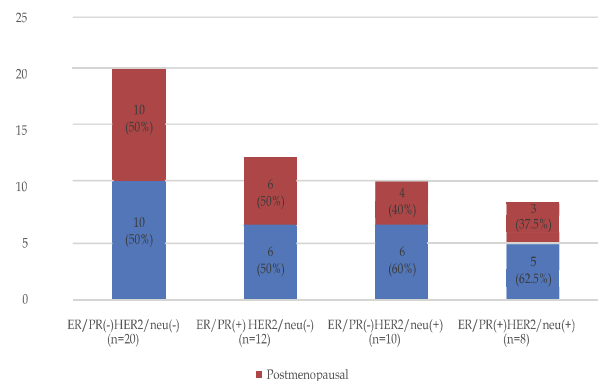
Table 4: Distribution of breast carcinoma cases according to scores of histologic grade (Nottingham Modification of the Bloom Richardson System)

	Tubule formation	Nuclear grading	Mitotic rate
Score 1	05(10%)	05(10%)	13(26%)
Score2	19(38%)	22(44%)	18(36%)
Score3	26(52%)	23(46%)	19(38%)
Total	50	50	50

Out of 20 cases of triple negative, 10 cases (50%) were premenopausal and 10 cases (50%) were post menopausal. Among 12 cases of ER/PR(+) HER2/neu(-), 6 cases (50%) were premenopausal and 6 cases (50%). Of the 10 cases of ER/PR(-) HER2/neu(+), 6 cases (60%) were premenopausal and 4 cases (40%) were post menopausal. Among the triple positive 3 cases (62.5%) were post menopausal and 3 cases (37.5%) were premenopausal (Graph 4).

Left side breast carcinoma was predominantly seen irrespective of ER, PR, Her2/neu expression. Tumour size between 2-5 cm was seen in majority of the cases irrespective of ER, PR and HER/2neu (Table 5)

Out of 50 cases, 20 cases were triple negative, among them 15 cases (75%) were IDC(NOS), 1 case (5%) was mucinous carcinoma, 1case (5%) was papillary carcinoma, 1case (5%) was secretory carcinoma and 1 case (5%) was ILC.



Graph 4: Relationship of ER, PR And HER2/neu with menopausal status

Table 5: Relationship of ER, PR and HER/2neu with tumour size

Tumour size (cm)	ER/PR(-)HER2/neu(-) (n=20)	ER/PR(+)HER2/neu(-) (n=12)	ER/PR(-)HER2/neu(+) (n=10)	ER/PR(+)HER2/neu(+) (n=8)
<2	0(0%)	2(16.66%)	2(20%)	0(0%)
2-5	16(80%)	8(66.67%)	7(70%)	5(62.5%)
>5	4(20%)	2(16.66%)	1(10%)	3(37.5%)

Among 12 cases of ER/PR(+) HER2/neu(-) 11 cases (91.67%) were IDC (NOS) and 1 case (8.33%) was mucinous carcinoma. Of the 10 cases of ER/PR(-)HER2/neu(+) all the 10cases (100%) were IDC(NOS).Among the triple positive cases all the 8 cases (100%) were IDC(NOS) (Table 6).

Among the 20 cases of triple negative, maximum were grade-II (10 cases, 50%) followed by grade-III (9cases, 45%) and minimum were grade-I (1 case, 5%). Of 12 cases of ER/PR(+) HER2/neu (-), maximum were grade-II (8 cases, 66.67%) followed by grade-I (4 cases, 33.33%) Among the 10 cases of ER/PR (-) HER2/neu (+), maximum were grade-III (8cases, 80%)followed by 2 cases (20%) grade-II. Among the triple positive maximum were grade-II (6 cases, 75%) followed by,1 case (12.5%) of grade-I and 1 case (12.5%)grade-III (Table 7).

Out of 20 cases of triple negative,8 cases (40%) showed positive lymph node status and 12 cases (60%)lymph node status was not available. Among 12 cases (24%) of ER/PR(+) HER2/Neu(-), 7 cases (58.33%) lymph node status was not available, 3 cases (25%) showed positive metastatic lymph node deposits and 2 cases (16.67%) showed no metastatic lymph node deposits. Of the 10 cases (20%) of ER/PR(-) HER2/neu(+), 6 cases (60%) showed positive metastatic lymph node deposits, 2 cases (20%) showed no metastatic lymph node deposits and 2 cases (20%) lymph node status was not available. Among triple positive cases, in 5 cases (62.5%), lymph node status was not available and 3 cases (37.5%) showed positive metastatic lymph node deposits (Table 8).

Table 6: Relationship of ER, PR and HER/2neu with histological subtype

Histological - subtype	ER/PR(-) HER2/neu(-) (n=20)	ER/PR(+) HER2/neu(-) (n=12)	ER/PR(-) HER2/neu(+) (n=10)	ER/PR(+) HER2/neu(+) (n=8)
IDC (NOS)	15(75%)	11(91.67%)	10(100%)	8(100%)
Mucinous	1(5%)	1(8.33%)	0(0%)	0(0%)
Medullary	1(5%)	0(0%)	0(0%)	0(0%)
Papillary	1(5%)	0(0%)	0(0%)	0(0%)
Secretory	1(5%)	0(0%)	0(0%)	0(0%)
ILC	1(5%)	0(0%)	0(0%)	0(0%)

Table 7: Relationship of ER, PR and HER/2 neu with histologic grade

Histologic Grade	ER/PR(-) HER2/neu(-) (n=20)	ER/PR(+) HER2/neu(-) (n=12)	ER/PR(-) HER2/neu(+) (n=10)	ER/PR(+) HER2/neu(+) (n=8)
I	1(5%)	4(33.33%)	0(0%)	1(12.5%)
II	10(50%)	8(66.66%)	2(20%)	6(75%)
III	9(45%)	0(0%)	8(80%)	1(12.5%)

Discussion

Breast cancer is a heterogeneous disease composed of growing number of recognized biological subtypes. Prognostic indicators based on currently available clinical and histopathologic variables such as tumor size, tumor grade, lymph node status and hormone receptor status already exist and are used to predict a patient's clinical outcome in certain situations [47]. It is well known that ER, PR and HER-2 represent the most acceptable factors for predicting prognosis response or resistance to treatment and the potential use of newer drugs [41]. Assessment of ER/PR and HER2 in breast cancer is mandatory in clinical practice and HER2/neu expression in various types of malignant breast lesions and correlated with tumor type, histological grade and lymphnode status. Our study comprised of 50 cases of malignant breast lesions.

In the present study mean age was in concordance with the study conducted by Nikhra. P et al. [48] and was less when compared to study by Ambroise et al. [47] Mean age of Indian breast cancer patients is found to be lower when compared to the western countries with an average difference of one decade [49].

In our study premenopausal cases were most common which was in concordance with the study of Patnayak.R et al. [54]

In the study conducted by Azizun-Nisa et al [51] and Ambroise et al [50] left side breast was most commonly involved which was concordance with our study

In the present study, 36 cases (72%) had the tumor size between 2-5 cms and was concordance with the study done by Muddawa L et al. [52] (74%) and was high when compared to other studies.

Our study showed, IDC as the most common type of breast carcinoma which was in concordance with other studies. In the present study grade II tumours were more common. Similar observations were made by Azizun-Nisa et al. [51], Patnayak.R et al. [54], Ambroise et al 49 (Table 9)

Table 8: Relationship of ER, PR and Her/2neu with Lymph Node Status

Lymph node Status	ER/PR(-) HER2/neu(-) (n=20)	ER/PR(+) HER2/neu(-) (n=12)	ER/PR(-) HER2/neu(+) (n=10)	ER/PR(+) HER2/neu(+) (n=8)
Positive	8(40%)	3(25%)	6(60%)	3(37.5%)
Negative	0(0%)	2(16.67%)	2(20%)	0(0%)
Not available	12(60%)	7(58.33%)	2(20%)	5(62.5%)

In the present study positive lymph nodes were seen in 40% of cases which was in concordance with the study of Onitilo et al. [55]. The present study 40% of ER & PR positive cases and 60% of ER & PR negative cases. Her2/neu positive cases were 36% and negative were 64% which was in concordance with other studies. (Table 10).

Our study showed maximum triple negative cases which was in concordance with study of Urmila Devi P et al. [56] but other studies showed less number of triple negative cases.

Conclusion

Breast carcinoma is a global disease with rising incidence in Indian women. In the present study invasive ductal carcinoma was most common histologic subtype. The interrelationship between ER, PR and HER-2/neu has an important role in the management of breast cancer. Endocrine therapy (tamoxifen) is recommended for tumors expressing ER/PR. Patients with breast carcinoma overexpressing HER-2 do not respond to tamoxifen therapy. Recently antiHER-2 antibodies (Herceptin) have been shown to be effective against HER-2 over expressing breast carcinomas.

We found the maximum triple negative cases (40%) in all subtypes indicating bad prognosis. Most of the triple negative cases were seen in the

age group 41- 50yrs. Maximum cases of triple negative breast cancers were grade II with lymph node metastasis. The lifestyle changes such as later age at marriage, reduced breast feeding, and westernization of diet may be associated with occurrence of breast cancer in younger population in India [55].

We conclude that the most common type of malignancy are triple negative, which are correlating by various authors, indicating bad prognosis.

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Table 10: Comparison of ER,PR and HER2/neu status in breast carcinoma with other studies

ER,PR and HER2 Status	Munjal.K et al71 (%)	Nikhra.P et al75(%)	Present study (%)
ER			
Positive	41.1	39.5	40
Negative	58.9	60.5	60
PR			
Positive	41.1	41.8	40
Negative	58.9	58.2	60
Her2/neu			
Positive	40.2	32.5	36
Negative	59.8	67.5	64

Table 9: Histological grading in comparison with other studies

Authors	Grade I %	Grade II %	Grade III %
Azizun-Nisa et al77	6.7	55.3	38
Patnayak.R et al80	3.9	60.9	35.2
Ambroise et al74	-	57.3	33.3
Present study	12	52	36

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