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Clinicopathological study of Breast cancer with special emphasis on IHC4 Assay

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Abstract

Background: *Breast Carcinoma has a high mortality and poor prognosis in Indian subcontinent owing to late presentation at advanced ages as well as hesitancy among the female population. Over the last few decades, more favorable survival of Ca breast cases due to newer diagnostic and treatment modalities. The IHC 4 assays of the breast panel includes immunohistochemical studies of Estrogen Receptor (ER), Progesterone receptor (PR), Human epidermal growth factor receptor (Her2/neu) and Ki67. IHC 4 assay is one of the most basic and cost effective immunohistochemical/ molecular profile that can be available to these patients in the remote facilities where higher modalities such as advanced molecular markers, next generation sequencing and epigenetic studies are unavailable.*

Materials & methods: *All the mastectomy specimens were examined in detail. Tissue fixation, grossing, tissue processing, staining and mounting was done. After detailed histopathological examination, tissue was subjected to IHC markers ER, PR, Her2/neu and Ki67. Receptors status and histopathological findings were then corelated with the clinical findings and node status of the patients.*

Results: *Maximum cases seen in 40 to 49 years age group. Larger lumps (>2 cm) were observed in 93% of the females with IDC Grade II as the commonest subtype. Triple negative receptor status and HER-2/neu expression was the commonest immune profile, showed association with increasing grade, large tumor size and lymph node involvement.*

Conclusion*: Early detection of Breast carcinoma and treatment with hormonal therapy can reduce mortality. Today the hormonal receptor, molecular classification and multi gene assays, next generation sequencing and epigenetics facilities present at higher centers are providing tailored therapy. Predictive and precision markers have improved outcome, if required treatment/ therapy is delivered at an appropriate time.*

Keywords: Breast cancer histopathology, IHC4Assay, Immunohistochemistry.

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I. Introduction

Breast Cancer (BCa) is the commonest malignancy in women worldwide, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases [1]. According to epidemiological studies, the global burden of BCa is expected to cross almost 2 million by the year 2030[2]. In India, the incidence has increased significantly, almost by 50%, between 1965 and 1985 [3]. The estimated number of incident cases in India in 2016 was 1,18,000 (95% uncertainty interval, 107000 to 130000), 98.1% of which were females, and the prevalent cases were 5,26,000 (4,74,000 to 5,74,000). Over the last 26 years, the age-standardized incidence rate of BCa in females increased by 39.1% (95% uncertainty interval, 5.1 to 85.5) from 1990 to 2016, with the increase observed in every state of the country [4]. In India, BCa accounted for 13.5% (1,78,361) of all cancer cases as per the GLOBOCAN data 2020 and 10.6% (90,408) of all deaths with a cumulative risk of 2.81**[5]**.

Breast Carcinoma has a high mortality and poor prognosis in Indian subcontinent owing to late presentation at advanced ages as well as hesitancy among the female population. Over the last few decades, more favorable survival of Ca breast cases due to newer diagnostic and treatment modalities. The IHC 4 assays of the breast panel includes immunohistochemical studies of Estrogen Receptor (ER), Progesterone receptor (PR), Human epidermal growth factor receptor (Her2/neu) and Ki67. IHC 4 assay is one of the most basic and cost effective immunohistochemical/ molecular profile that can be available to these patients in the remote facilities where higher modalities such as advanced molecular markers, next generation sequencing and epigenetic studies are unavailable. Also, these basic investigation acts as prognostic and predictive markers that

helps in institution of proper therapy to patients. The IHC determination of breast cancer subtype with regard to ER, PR and HER2/neu status can contribute to improved selection of therapy and prognosis.

II. Aims And Objectives

1.To study clinicopathological correlation in patients with Carcinoma breast.
2. To study expression of IHC4 Assay (ER, PR, HER2/neu and Ki67 markers).
3. To study the molecular subtypes of Carcinoma breast depending upon the receptor status.

III. Material And Methods

This was a prospective study done in Department of Pathology of Dr. Vaishampayan Memorial Government Medical College, Solapur, Maharashtra, India for a period of 6 months from August 2018 to January 2019. Total sample size was 30 cases. It includes all the BCa patients whose mastectomy specimens received in the department of pathology. It excludes patients with small biopsy, inadequate sample, poorly preserved samples and over and under fixed specimens (if any).

Fixation was done according to ASCO/CAP guidelines for 6 to 72 hours. Over fixation and under fixation was kept in mind, as it hampers IHC results. After fixation all the specimens were examined and grossed in detail. The representative bits were then taken and submitted for tissue processing. Routine paraffin embedding was followed by microtomy where 4-micron thin sections were cut and were subjected to Deparaffinization, Rehydration and Hematoxylin & Eosin staining (H&E) (hematoxylin stain, differentiation, bluing, eosin staining). The slides were then dehydrated using ascending grades of alcohol, dipped in xylene and mounted with DPX. Detailed histopathological examination with assessment of tumour was done along with proliferative index and tumour grading using Modified Bloom-Richardson (MBR) grade.[6]

For Immunohistochemistry (IHC 4 Assay) staining (ER, PR, HER2/neu and Ki67) was done using peroxidase-antiperoxidase (PAP) Method. For IHC, 4-micron thin sections were cut with microtome, which were then deparaffinized and rehydrated. Antigen retrieval was done by microwaving and endogenous peroxidase activity was blocked by 0.5% H_2O_2. Slides were then incubated overnight at +4 °C in a refrigerator with the primary antibodies of appropriate dilution. The reaction was visualized by the Elite ABC Kit for ER, PR and by the Envision kit for HER2. Positive controls were stained in similar manner while in Negative Control primary antibody, isotype control or absorption control were omitted. [6]

The results are then interpreted. Based on the nuclear staining, tissue samples were classified as positive for ER and PR even if 1% of the tumor cells showed positive nuclear staining whereas for HER2 neu hormonal receptor membrane staining is important. In order to find out proliferative index of the tumor, Ki-67 was done. Tumor was considered positive, tumor cells showed positive stained nuclei. It was noted that Ki-67 is <20% or more than 20%. The Molecular classification of each case was done based on these parameters.

Modified Bloom-Richardson (MBR) grading has been done which is a pathologic grading system for breast cancer that has been shown to have prognostic significance in patients with node-negative disease**.** Reporting done as per ER/PR scoring system (Modified Allred scoring system) as per ASCO/CAP Guidelines[7,8] [Table 1]. Her2 scoring was done according to the guidelines given from the American Society of Clinical Oncology (ASCO) shown in [Table 2]. FISH is required for equivocal Her2/neu positivity. Hence, Her2/neu 2+ was taken as negative along with her2/neu 0 and 1+. Only 3+ on IHC was taken as positive.

Statistical analysis: Chi-square test was used to determine the statistical significance between ER/PR status and Her2/neu status correlation with various parameters was done such as patient's age, axillary lymph node status, tumor size, and tumor grade with respect to invasive ductal carcinoma breast. A value of $P < 0.05$ was considered as statistically significant.

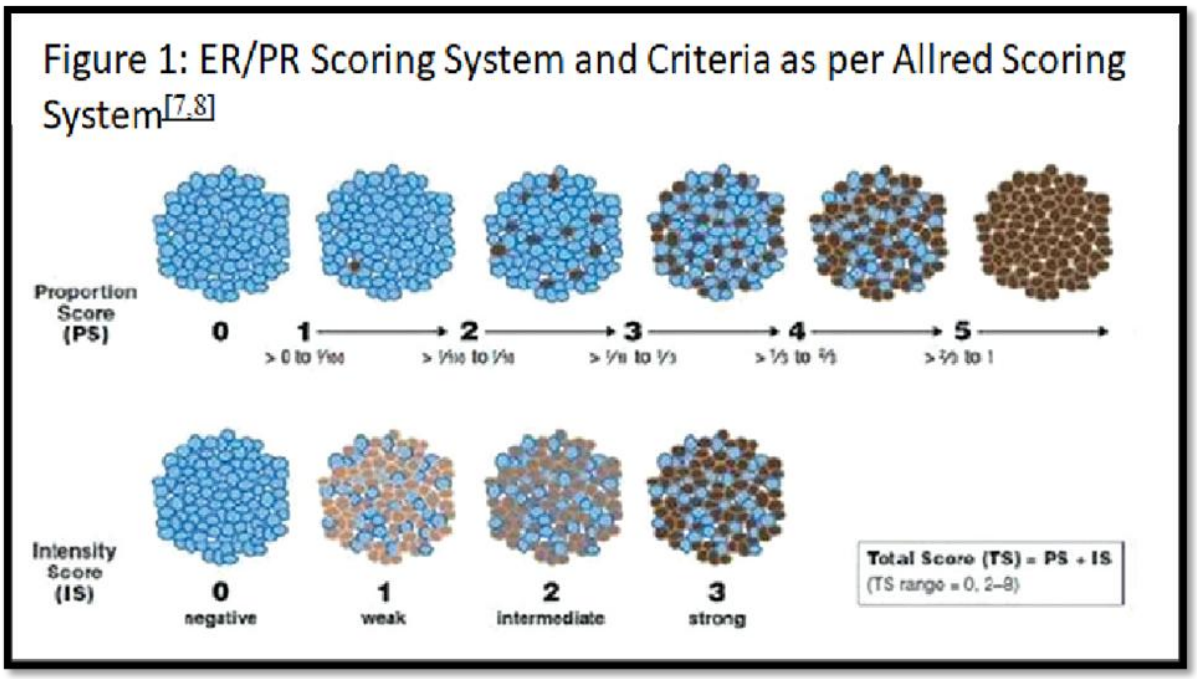


Table 1: Modified Allred Scoring for ER and PR as per ASCO/CAP Guidelines [7,8]

Proportion Score	
Score	*Percentage of stained cells*
0	No cells are ER positive
1	≤1% cells are ER positive
2	1-10% cells are ER positive
3	11-33% cells are ER positive
4	34-66% cells are ER positive
5	67-100% cells are ER positive
Intensity Score	
Score	*Intensity of staining*
0	Negative
1	Weak
2	Intermediate
3	Strong
Allred Score (Allred score=Proportion Score + Intensity Score)	
Allred score	*Effect of hormone therapy*
0-1	No effect
2-3	Small (20%) chance of benefit
4-6	Moderate (50%) chance of benefit
7-8	Good (75%) chance of benefit
ER: Estrogen receptor; PR: Progesterone receptor	

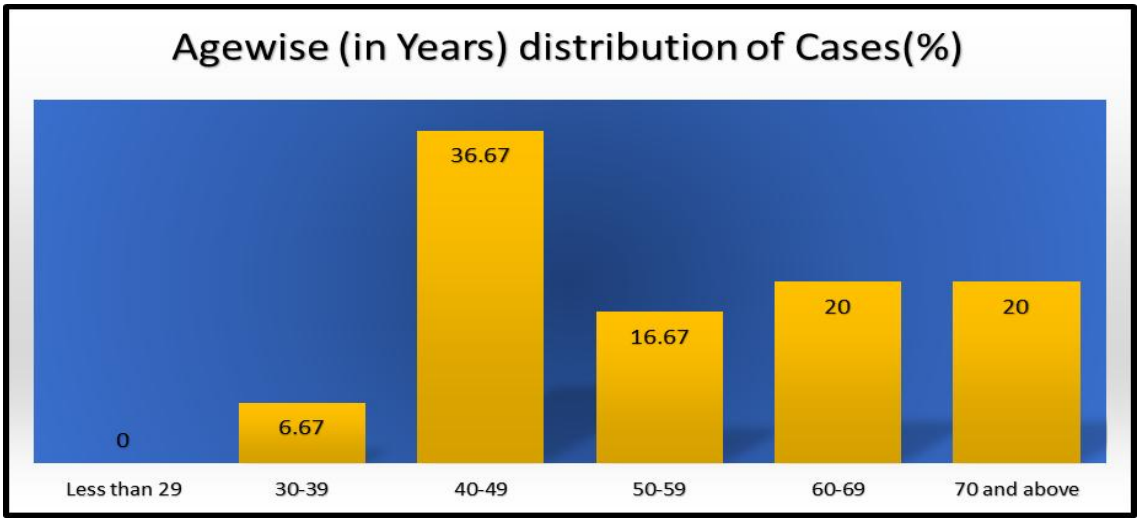
Table 2: Human epidermal growth factor receptor (Her2/neu) scoring system and criteria according to the ASCO/CAP [8,9]

Score to Report	HER2 Overexpression	Assessment of Protein Staining Pattern
0	Negative	No staining is observed, or membrane staining in fewer than 10% of tumor cells.
1+	Negative	A faint or barely perceptible membrane staining is detected in more than 10% of tumor cells. The cells are only stained in part of the membrane
2+	Borderline	A weak to moderate complete membrane staining is observed in more than 30% of tumor cells.
3+	Positive	A strong complete membrane staining is observed in more than 30% of the tumor cells.

HER2: Human epidermal growth factor receptor 2

IV. Results And Discussion

Table 3: Age wise distribution of Breast Cancer cases (%) in present study



Out of the total 30 cases, the age range varies from 30 years to 90 years. Mean age is 60 years. The most common age group affected is 40 – 49 years (36.67%) followed by 60–69 year age group and 70 years and above age group comprising 20% each (Table 3). Similar results were seen in a study by Ranjan et. al [11] in which the most common age group affected was 41-50 years followed by 51-60 years and in a study of Clegg-Lamptey et. al [12] where the most common age group affected was 40-44 years followed by 45-49 years. In the present study of the total cases, 96.67% cases are females (Table 4) where as 3.33% are males which were comparable to the studies of Hussain et.al [13] where 96.6% cases were females and 3.4% were males affected with Breast cancer.

Table 4: Gender wise distribution of Breast Cancer cases (%) in present study

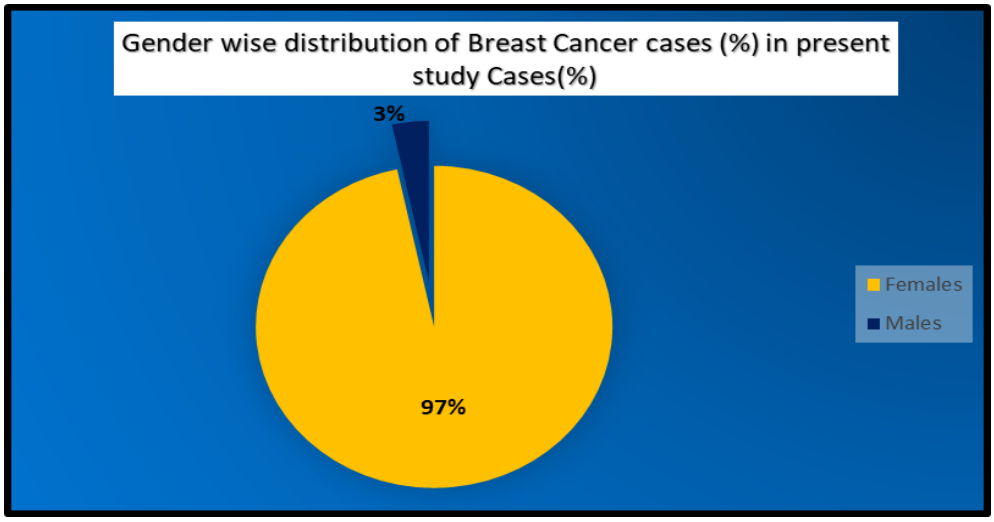
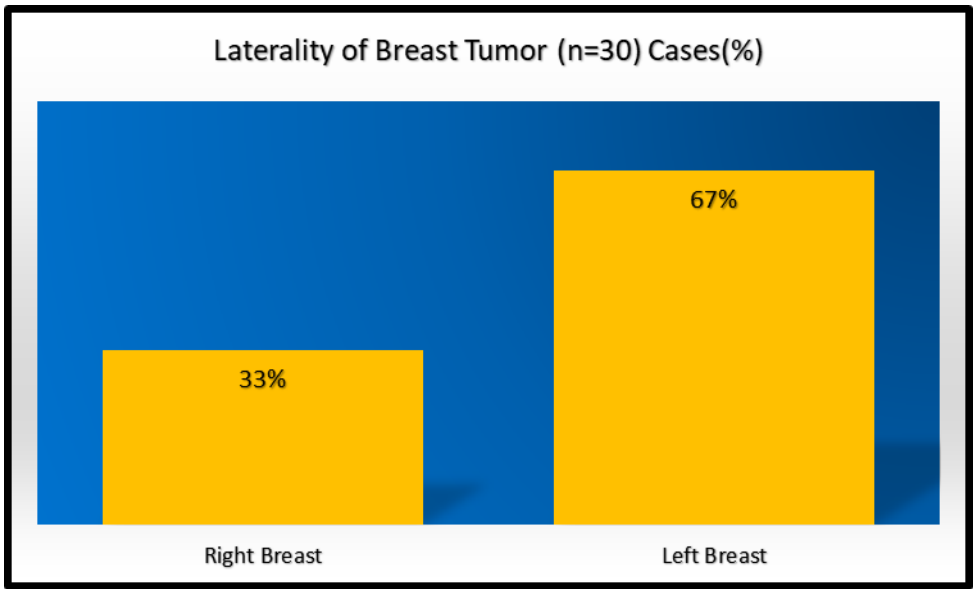


Table 5: Laterality of Breast Cancer cases (%) in present study



The most common site of the tumor is left breast (Table 5) comprising 67% of the cases and right breast in 33% cases. It is comparable to studies done by Cheng et. al [14] (except for Invasive mucinous and invasive medullary cancer) and studies of Trichopoulos et. al. [15]

The most common quadrant occupied by tumor is upper outer quadrant (66.6%) in our study followed by upper inner (13.3%) and central quadrant (10%) and is comparable to the studies of Lee et.al [16]. Also in a study by Rummel et. al, it is stated that 51.5% cases showed tumor location in upper outer quadrant which was significantly higher than other quadrant [UIQ (15.6%)> LOQ (14.2 %)> Central (10.6 %)> LIQ (8.1%)]. [17] In study of Chan S et. al[18], 60.9% cases of breast carcinoma had mass in upper outer quadrant followed by 14.5% in upper inner, 6.4% in lower outer and 18.2% in lower inner quadrant which is comparable to the findings in the present study. Tumor size is compared in all the 30 cases and it was found that 93.3% cases have tumor size of more than 2 cm and 6.67% cases have tumor size less than 2 cm (Table 6) and is comparable with the studies of Moses Ambroise et al, Azizun et al and Vaidyanathan et al.

Of the total cases, the most frequently encountered entity is Invasive ductal carcinoma (IDC) comprising of 90% of the total cases followed by 6.67% cases of Medullary carcinoma breast and 3.33% cases of Mucinous carcinoma (Table 7, Figure 2). Our studies were very much comparable to the studies of most of

the authors in Table 8. Modified Bloom Richardson grading was done in all the 27 cases of IDC, of which Grade II (77.7 %) was the most common grade followed by Grade I (14.11%) and the least common was Grade III (Table 9) which was comparable to the studies of Siadati et al and Biswal et al. (Table 10). In order to find out proliferative potential of the tumor, ki 67 was done. Of total 30 cases, Ki67 was more than 20% in 70% cases.

Table 6: Comparison of Size of Tumor in Present study

Authors	Taucher et al [19]	Onitilo AA et al [20]	Vaidyanathan et al[21]	Azizun et al [22]	Moses Ambrose et al [23]	P. Biswal et al [24]	Present Study
Year & Tumour size	(2003)	(2009)	(2010)	(2011)	(2014)	(2015)	(2018)
More than 2cm	40.1%	29.0 %	88.8 %	88.0 %	91.5 %	86.0 %	93.3%
Less than 2cm	59.9%	71.0%	11.2%	12.0%	8.5%	14.0%	6.67%

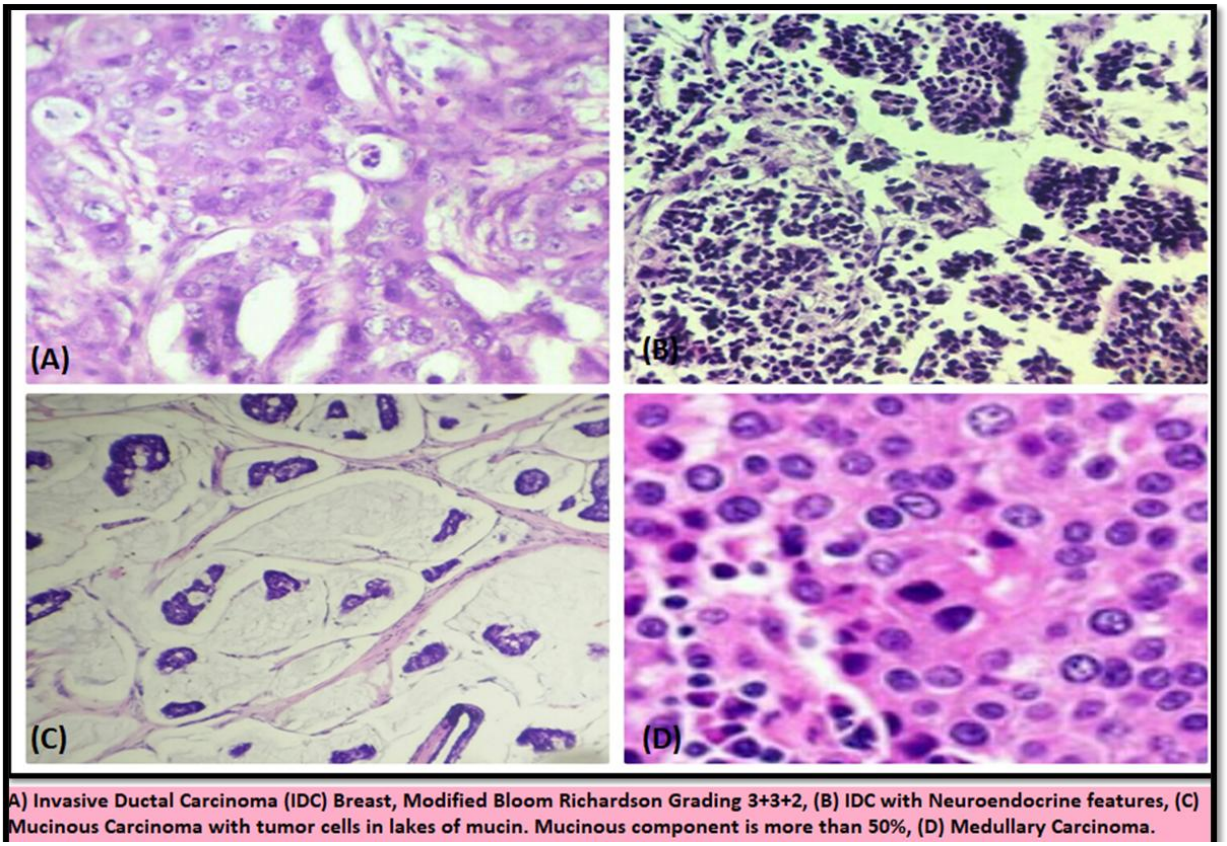
Table 7: Histopathological Analysis of Ca Breast (n=30)

Histopathological Diagnosis	No. of cases	Percentage
Invasive Duct Carcinoma	27	90.0%
Medullary Carcinoma	2	6.67%
Mucinous Carcinoma	1	3.33%
Total	30	100

Table 8: Comparison of Frequency of Infiltrating ductal Carcinoma (IDC)

Author	Bane et al [25]	Mudholkar et al [26]	Ashraf A. et al [27]	Gangadharan SGD et al [28]	Naveen Alexander et al [29]	Present Study
Year	(2007)	(2012)	(2014)	(2016)	(2017)	(2018)
Frequency of IDC (%)	85 %	88 %	95.44 %	94 %	84.4 %	90.0%

Figure 2: Histopathological types of Ca Breast in present study



A) Invasive Ductal Carcinoma (IDC) Breast, Modified Bloom Richardson Grading 3+3+2, (B) IDC with Neuroendocrine features, (C) Mucinous Carcinoma with tumor cells in lakes of mucin. Mucinous component is more than 50%, (D) Medullary Carcinoma.

Table no 9: Modified Bloom-Richardson Grading (n=27)

Modified Bloom-Richardson Grade	No. of Cases	Percentage
Grade I	4	14.11%
Grade II	21	77.7 %
Grade III	2	7.41 %
Total	27	100

Table no 10: COMPARISION OF HISTOLOGICAL GRADE OF VARIOUS STUDIES

Author	Doval et al 2015 [30]	Siadati et al 2015 [31]	Biswal et al 2015 [24]	Present Study 2018
Grade II	46.7%	80.66%	70%	77.7%

Table no 11: LYMPH NODE INVOLVEMENT (n=30) [n= Total no. of cases]

Lymph node status	No. of Cases	Percentage
Node Positive	18	60
Node Negative	12	40
Total	30	100

Table no 12: LYMPH NODE STATUS

Author	Rao et al 2013 [32]	Doval et al 2015 [30]	Ashraf et al 2014 [27]	Biswal et al 2015 [24]	Present Study 2018
Positive	**47.6%**	**52.9%**	**70.96%**	**27%**	**60%**
Negative	**52.4%**	**47.1%**	**29.04%**	**73%**	**40%**

All the lymph nodes were dissected and examined. The total number of lymph nodes dissected ranges from 12 in number (minimum) to 38 in number (maximum) with a mean of 25, lymph nodes were involved in 18 out of 30 cases attributing to 60% of node positive cases and 40% of node negative cases (Table 11). The studies were comparable to the studies of Ashraf et al and Doval et al (Table 12).

All the cases were classified as per Molecular Classification into Luminal, Her2/neu enriched and Basal subtype (Table 13). 30% cases were classified as Luminal, 23.33% as Her2/Neu enriched and 43.34% cases as Basal Like (Triple Negative). Basal Like is the most common type in the present study. Receptor study was inconclusive in one case. Receptor hormonal status was compared with the other studies (Table 14). 03 cases were both ER and PR positive (10.34%).

Figure 3: Expression of ER, PR, HER-2/neu Markers

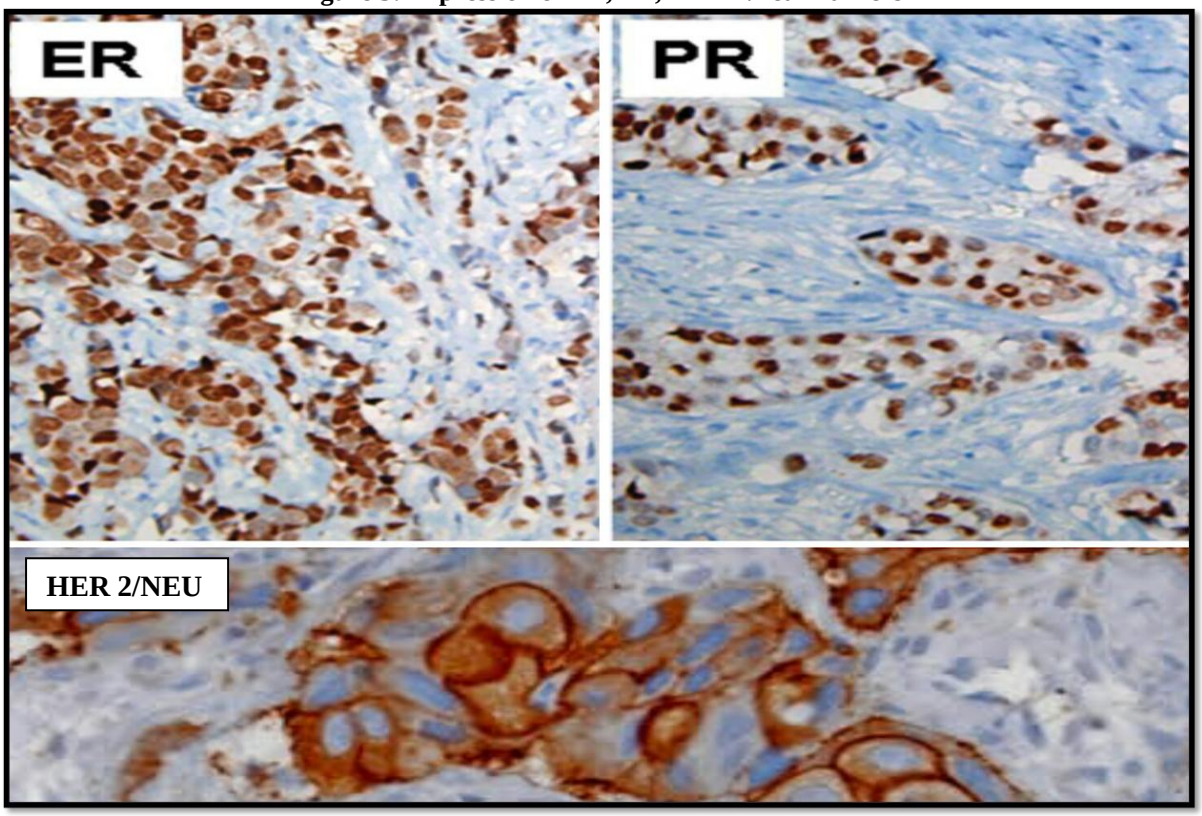


Table no 13: Classification of Ca Breast as per Molecular Subtype (n=30)

Molecular Subtype	No. of Cases	Percentage
Luminal (ER and/or PR positive, HER2/neu positive or negative)	**9**	**30.00**
HER-2/neu Enriched (ER negative, PR negative, HER2/neu positive)	**7**	**23.33**
Basal Like (Triple Negative)	**13**	**43.34**
Inconclusive	**1**	**3.33**
Total	**30**	**100**

Table no 14: Hormonal status

Hormone receptor	Rao et al 2013 [32]	Doval et al 2015 [30]	Biswal et al 2015 [24]	Present Study 2018
ER +ve	36.5%	62.2%	45%	27.59%
PR +ve	31.7%	53.2%	35%	13.79%
HER-2/neu	2.4%	23%	30%	24.14%
Triple negative	50%	23.8%	35%	44.83%

Table 15: Correlation of ER, PR, HER-2/neu predictive markers with clinico-pathological features

Grade	ER +ve	ER -ve	PR+ve	PR-ve	HER2/Neu +ve	HER2/Neu -ve	Triple negative
Grade I (n=4)	4 (100%)	0 (0%)	1 (25%)	3 (75%)	0 (0%)	4 (100%)	0 (0%)
Grade II (n=21)	1 (4.7%)	20 (95.2%)	3 (14.3%)	18 (85.7%)	6 (28.5%)	15 (60%)	12 (57.1%)
Grade III (n= 2)	1 (50%)	1 (50%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	0 (0%)
Total (n=27)	6	21	4	23	7	20	12

** Total number of cases do not match as one case had more than one finding.
*Receptor study was inconclusive in one case.
** Total number of cases do not match as one case had more than one finding.

Table 16: Correlation of ER, PR, Her-2/neu predictive markers with clinicopathological features

All the histopathological and immunohistochemistry parameters are corelated with the clinical findings

Predictive markers	ER +ve	ER -ve	PR+ve	PR-ve	HER2/Neu +ve	HER2/Neu -ve	Triple negative
Size <2 cm, n= 2	1 (50%)	1 (50%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	1 (50%)
≥2 cm, n= 27	7 (25.9%)	20 (74%)	4 (14.8%)	23 (85.1%)	7 (25.9%)	20 (74%)	12 (44.4%)
Node +ve n= 18	3 (16.6%)	15 (83.3%)	2 (11.1%)	16 (88.8%)	4 (22.2%)	14 (77.7%)	10 (55.5%)
Node -ve n= 11	5 (45.5%)	6 (54.5%)	2 (18.1%)	9 (81.8%)	3 (27.2%)	8 (72.7%)	3 (27.2%)

(Table 15& 16). Of the total Grade I (4 cases) tumors, 100% cases are positive for estrogen receptors, one is positive for progesterone receptor and 100% were negative for Her/2 neu. Out of 21 cases classified as grade II, 12 cases (57.1%) were triple negative. Among 2 cases of grade III tumor, 50% cases were ER positive and Her2/neu positive each.

Immunohistochemical profile is corelated with the tumour size as well as node positive and negative status. Tumor less than 2 cm in size, 50% are ER positive and 50% cases are triple negative. Tumor more than ≥2 cm size, 44.4% cases reported as triple negative, and 25.9% are Her2/neu positive. Out of total 18 node positive cases, 55.5% were triple negative, 22.2% were Her2/neu positive, 16.6% are Estrogen receptor positive and 11.1% progesterone receptor positive. It is also observed that more than 80% of node positive cases are estrogen and progesterone receptor negative. Out of 11 node negative cases, 45.5% cases were estrogen receptor positive followed by 27.2% cases positive for Her2/neu and triple negative.

V. Summary And Conclusion

1. Maximum cases seen in 40 to 49 years age group, as against above 50 years in western world.
2. Larger lumps (>2 cm) were observed in 93% of the females. This can be attributed to late medical consultation.
3. IDC with Grade II is the commonest subtype.
4. In present study, Triple negative receptor status and HER-2/neu expression was the commonest immunoprofile. This showed association with increasing grade, large tumor size and lymph node involvement.
5. These tumors are less susceptible to conventional hormonal therapy. Detecting and treating these tumors with triple negative status and Her2/neu expression is a challenge to reduce mortality.
6. The best choice is to accelerate the campaigning of breast cancer awareness and educate the women for self-breast examination and seeking early medical consultation in case if any abnormality noted.
7. Today, the hormonal receptor, molecular classification and multi gene assays, next generation sequencing and epigenetics facilities present at higher centers are providing tailored therapy. Predictive and precision markers have improved outcome, if proper therapy is delivered at proper time.
8. If this is fulfilled, the **Endocrine Harmony** of breast will remain in place.

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