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Original Research Article

Immunohistochemical Expression of ER, PR, and HER2/Neu in Breast Carcinoma -A Cross Sectional Study

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ABSTRACT

BACKGROUND
Breast carcinoma is a heterogeneous disease characterized by distinct molecular subtypes that influence treatment strategies and patient outcomes. This study explores the significance of immunohistochemical (IHC) profiling in breast carcinoma, focusing on key biomarkers: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/Neu).

OBJECTIVE
To find the correlation of ER, PR and Her2/neu IHC markers with clinical features, tumour size, lymph node involvement and histopathological grade.

METHODS
A three year prospective observational cross sectional study was conducted in the Department of Pathology, in integration with Department of General Surgery and Radiotherapy at Rangaraya Medical College, Kakinada, Andhra Pradesh, India. A total of 55 cases of breast carcinoma were included in the study. The histopathological grading of the breast carcinoma was done according to the Nottingham modification of the Bloom Richardson grading system. All the cases underwent immunohistochemistry for ER, PR and Her2neu expression. Correlation of ER, PR and Her2neu with various prognostic factors was done.

RESULTS
The study included a total of 55 cases, out of which 22/55 (40%) were below 40yrs and 33/55 (60%) were above 40yrs. 60% of the tumors were in the size range of 2-5 cm (T2). 34.5% of the tumors belong to N2 category and 58.2% of the tumors were of high grade. 43.6% of the patients fall under Stage IIIA group. 78.2%) were of Invasive Ductal Carcinoma of No Special Type (NST). About 51% of the patients were ER/PR negative. Half of the patients (51%) were Her2/neu negative (Grade I (0/1+)), 25.5% were Her2/neu positive (Grade III) and 23,6 % were unequivocal.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 08, 2024

CONCLUSION
Our study contributes to the body of evidence regarding histological grading and receptor expression in breast cancer within Asian populations. The observed lower rates of ER and PR positivity, alongside the relatively high prevalence of Grade III tumors, highlight the need for ongoing research to understand the factors influencing these trends and to optimize treatment strategies tailored to specific patient demographics.

KEYWORDS
Breast Carcinoma, Estrogen receptor, Progesterone receptor, Human epidermal growth factor receptor

Introduction
Breast cancer is the most common cancer occurring in women and is the second leading cause of cancer-related deaths among women.[1] In the spectrum of symptoms related to breast disease, breast lump is the most common clinical presentation among the spectrum of breast related symptoms. Sensitive method to detect BC is "Triple assessment" which includes three modalities, clinical examination, imaging (mammogram and/or ultrasound) and biopsy (FNAC and core biopsy).[2,3] In a case of breast carcinoma, along with tissue diagnosis, determining hormonal receptor status and molecular subtypes is crucial to initiate appropriate therapy.[4]

The patient's outcome can be anticipated through prognostic factors. These prognostic factors can be used to estimate the response to therapy and some are useful as predictive factors. Factors that are both prognostic and predictive include estrogen receptor (ER) and progesterone receptor (PR) status and human epidermal growth factor receptor (Her2neu).[5]

Immunohistochemistry serves as a vital tool for assessing the expression of these biomarkers, aiding in the molecular classification of breast tumours into subtypes such as luminal A, luminal B, HER2-enriched, and triple-negative breast cancer.[6] ER and PR positivity correlates with responsiveness to hormone therapies such as tamoxifen, while HER2/neu overexpression indicates a potential benefit from targeted therapies like trastuzumab but not to endocrine-based therapies. However, histological type of tumour, number of involved lymph nodes, size of the tumour, tumour grade and the patient's age are independent prognostic factors.[7,8,9]

This study explores the significance of immunohistochemical (IHC) profiling in breast carcinoma, focusing on key biomarkers: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2 Neu).

Objectives
To evaluate immunohistochemical expression of ER, PR and Her2/neu in breast carcinoma cases and to correlate with clinico-histopathological features like type, grade and stage of tumor.

MATERIAL AND METHODS
Study Design
A three year prospective observational cross sectional study was conducted in the Department of Pathology, in integration with Department of General Surgery and Radiotherapy at Rangaraya Medical College (RMC), Kakinada, Andhra Pradesh, India. We received a total of 350 cases of modified radical mastectomy specimens and IHC was performed for 55 breast carcinoma cases.

Inclusion Criteria
1. Lumpectomy specimens

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2. Mastectomy specimens

Exclusion Criteria
1. Trucut biopsy specimens
2. Blocks received from outside
3. IHC reports done elsewhere other than the institute

Data Collection
Demographic and clinical data of the cases was collected from histopathology request forms.

Methods
All the specimens were fixed in neutral buffered formalin for 8-10 hours. Tumor size was assessed during the grossing after fixation. Adequate tumor tissue and all the lymph nodes identified were processed according to standard grossing protocol by Tata Memorial Hospital.

Formalin fixed paraffin embedded tissue blocks were processed for haematoxylin and eosin staining. Appropriate block containing adequate amount of tumor tissue and internal controls were selected for immunohistochemical staining. IHC was performed according to standard protocol manually and also by BIOGENEX staining system (i6000) of Biogenex Laboratories Inc; CA, USA.

Tumor type was categorized. Tumor grade was assessed on H&E stained slides using the Nottingham Grading system. This grading system includes 3 components – tubule formation, nuclear pleomorphism and mitotic count. Each variable was given a score 1 to 3 and the scores were added to produce the grade.

Tubule formation and nuclear pleomorphism were assessed by interpreting 3 to 5 blocks of tumor tissue according to tumor size.

Mitotic score was determined by the number of mitotic figures found in 10 consecutive high power fields in the most mitotically active part of the tumor.

Histological grade was assigned as per the Nottingham grading system [Table-1]**.**

Histopathological features	**Score**		
	1	**2**	**3**
Mitotic activity (per 10 hpf, field diameter 0.56mm)	≤8	9-17	≥18
Nuclear pleomorphism	Mild	Moderate	Marked
Tubule formation (%)	>75	10-75	<10

Table 1: The Nottingham modification of the Bloom Richardson grading system

Immunohistochemistry
IHC was done using the Peroxidase - Antiperoxidase method. The analysis for the expression of ER/PR receptors and HER2 expression was done by using an antibody as per the standard procedure [table-2]. The scoring of the stained sections was done by two independent observers and the average value was taken as the expression [Table-3,4].

IHC Marker	**Antibody**	**Immunogen**	**Clone**	**Species**	**Protein concentration**
ER	Anti-Estrogen antibody (AM 272-2ME)	Recombinant estrogen receptor protein	1D5	Mouse	~50 mg/ml
PR	Anti-Progesterone Receptor Antibody (AM328-5ME)	Purified human progesterone receptor protein	PR88	Mouse	~10-15 mg/ml

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 08, 2024

Her2/neu	Anti-HER-2/neu Antibody (AN471/5ME)	Synthetic peptide corresponding to internal domain of the c-erB-2 Protein (HER-2/neu)	CB11	Mouse	~10-15 mg/ml
Table 2: Reagents used for the IHC markers					

Interpretation

Allred scoring system was used for ER and PR (a negative result was defined as a score of 0 or 2 and positive between 3- 8) (Table 2). Her2neu scoring of IHC slides was done as per the ASCO/CAP 2018 guidelines (American Society of Clinical Oncology and the College of American Pathologists) and cases were classified into scores of 0, 1+, 2+ and 3+ (Table 3).

Allred scoring system

Proportion Score	Proportion of positive staining nuclei (%)	Intensity Score	Average intensity of positively stained nuclei
0	0	0	None
1	<1	1	Weak
2	1-10	2	Average
3	11-33	3	Strong
4	34-66%		
5	≥67%		
Allred score= Proportion score +Intensity scores (0-8)			
Table 3: Allred score guidelines for ER and PR evaluation			

Score	Her2 protein assessment	Staining pattern
0	Negative	No staining or membrane staining in <10% of invasive tumor cells
1+	Negative	Faint/barely perceptive membrane staining detected in >10% of invasive tumor cells
2+	Equivocal	Weak to moderate complete membrane staining in >10% of invasive tumor cells or <30% with strong complete membrane staining
3+	Positive	Strong complete membrane staining in >30% of invasive tumor cells
Table 4: Allred score guidelines for HER2/Neu evaluation		

Statistical analysis

The collected data were entered in Microsoft Excel worksheet and the results were compiled and analyzed statistically using SPSS-16 and were expressed as number and percentage.

Ethical consideration and permission

Ethical clearance was obtained from the Institutional ethical committee.

Results

The study included a total of 55 cases, out of which 22/55 (40%) were below 40yrs and 33/55 (60%) were above 40yrs.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 08, 2024

Distribution of Age	No. of persons
19- 29	1 (1.8%)
30-39	13 (23.6%)
40-49	24 (43.6%)
50-59	6 (11%)
60-69	5 (9.1%)
70-79	5 (9.1%)
80-89	0 (0%)
90-99	1 (1.8%)
TOTAL:	55 (100%)

Table 5: Distribution of age (n=55)

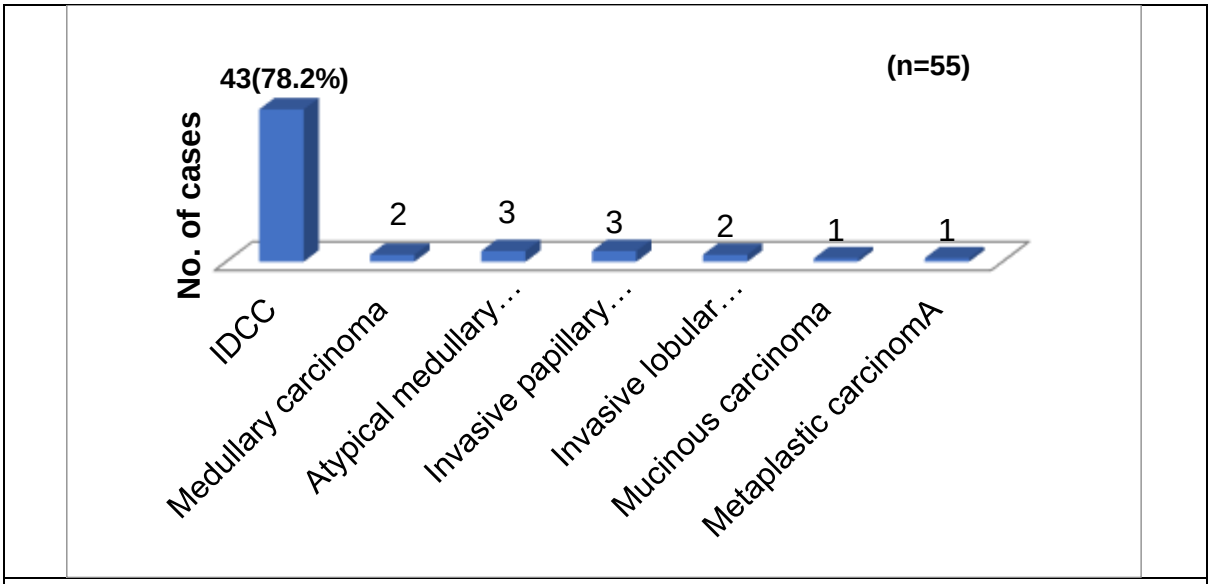

Fig 1. Histological Type of tumor

Most of the cases (78.2%) were of Invasive Ductal Carcinoma of No Special Type (NST).

Tumor size (T)	No. of persons (%)	Lymph Node Involvement (N)	No. of persons (%)	Distant Metastasis (M)	No. of persons (%)
T1 (≤2cm)	3 (5.5%)	N0	15 (27.3%)	Mx	0
T2 (2-5cm)	33 (60.0%)	N1 (1-3)	16 (29.1%)	M0	0
T3 (>5cm)	19 (34.5%)	N2 (4-9)	19 (34.5%)	M1	0
T4	0 (0%)	N3 (≥10)	5 (9.1%)	-	-
Total	**55**	**Total**	**55**	**Total**	**0**

Table 6: Distribution of cases based on tumor size, lymph node status and distant metastasis

60% of the tumors were in the range of 2-5cm size (T2). Most of the tumors (34.5%) belong to N2 category. None of the tumors showed distant metastasis.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 08, 2024

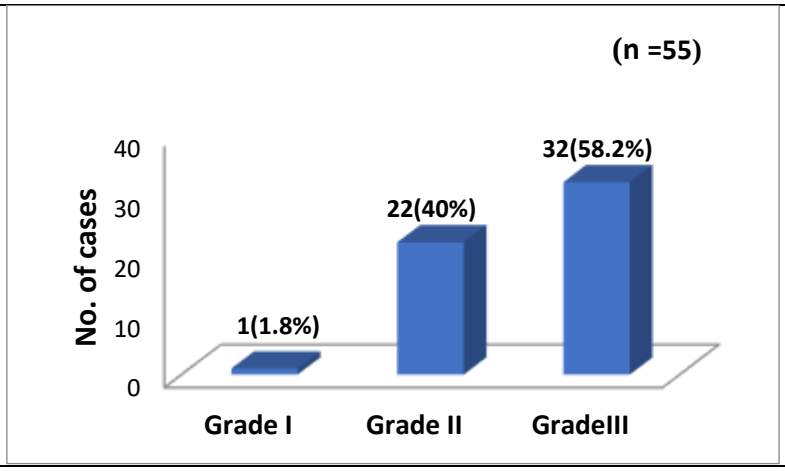

Fig 2: Tumor Grade by Nottingham grading

Majority of the tumors (58.2%) were of high grade.

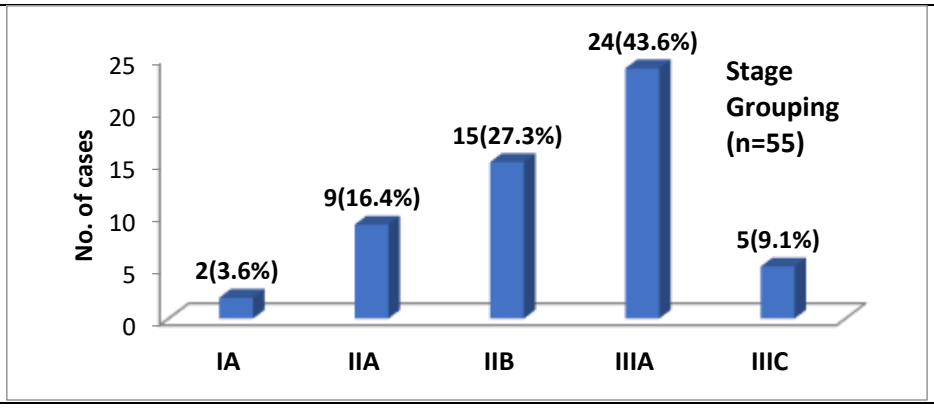

Fig 3. Tumor Stage Grouping

Tumor staging was done according to AJJCC/UICC TNM classification (WHO 2012). 43.6% of the patients fall under Stage IIIA group.

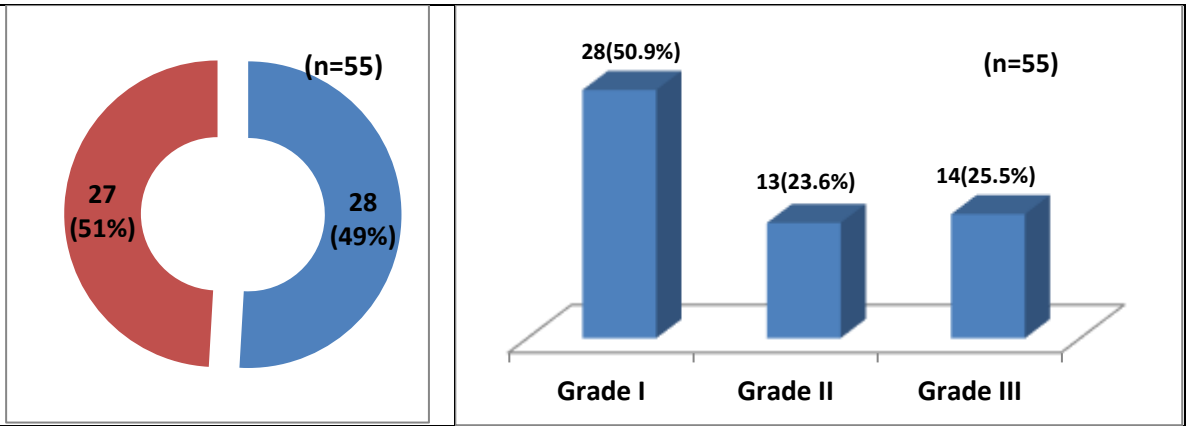

Fig 4. ER/PR and HER2/Neu expression

About 51% of the patients were ER/PR negative. Half of the patients (51%) fall under Grade I (0/1+), 25.5% were of Grade III and 23, 6 % were unequivocal.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 08, 2024

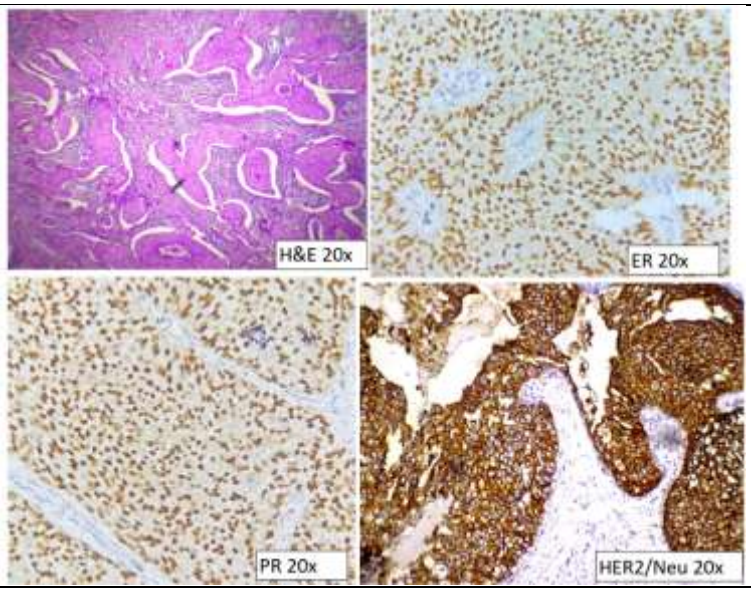

Fig 5: Breast Carcinoma showing strong ER, PR and HER2neu expression

Tumor Grade	ER/PR Positive	ER/PR Negative	HER2 Grade 1	HER2 Grade 2	HER2 Grade 3
I (n= 1)	0	0	0	0	0
1.8%	1	0	1	0	0
II (n=22)	5	2	3	2	2
40%	6	9	7	2	6
III (n=32)	11	8	9	7	3
58.2%	4	9	8	2	3
n= 55	27(49%)	28(51%)	28(51%)	13(24%)	14(25%)

Table 8: Correlation of Tumor grade and ER/PR and HER2/NEU markers

DISCUSSION

Breast cancer is a complex disease characterized by various histological grades and receptor expression profiles that significantly impact treatment decisions and prognostic outcomes.[10,11] The most common histological type is IDC-NST. The findings from our study, which observed that Histological Grade III tumors constituted 58.2% of cases, followed by Grade II (40%) and Grade I tumors (1.8%), align with trends reported by Desai et al., and Azizun et al.,[12,13] The predominance of higher-grade tumors suggests a more aggressive disease profile within our study population, which could be attributed to various factors, including environmental influences, genetic predisposition, and differences in healthcare access.

Comparison of Hormone Receptor Positivity

Our findings highlight a concerning trend in hormone receptor positivity rates among Asian populations[14,15] which tend to be lower than those reported in Western countries, where more than 50% of tumors express hormone receptors (Mudduwa et al.).[16] The following comparisons of estrogen receptor (ER) and progesterone receptor (PR) positivity among different Asian studies illustrate this trend:

STUDY	ER positivity	PR positivity
Shet et al.,[14]	52.4%	45.2%
Lakmini et al.,[15]	45.75%	48.3%

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 08, 2024

Desai et al.,[12]	32.6%	46.1%
Azizun et al.[13]	32.7%	25.3%
Our study	29.5%	28.7%

Table 9: Comparison of receptor positivity with other Asian studies

Our study's ER positivity of 29.5% and PR positivity of 28.7% is notably lower than many other Asian studies, indicating a potential shift in the biological behavior of breast tumors in this region or highlighting the need for further investigation into the underlying causes of such discrepancies. The declining trend in receptor positivity may also reflect differences in patient demographics, lifestyle factors, and environmental exposures that warrant more comprehensive research.

HER-2/neu Expression Analysis

In terms of HER-2/neu expression, our study revealed that 28 out of 55 cases (51%) showed Grade I expression. This finding raises important considerations regarding the methods of evaluation and scoring criteria employed. The variability in HER-2/neu expression may stem from the choice of antibodies used in immunohistochemistry, the specificity of the assay, or inherent differences within the studied populations.

HER-2/neu status plays a crucial role in guiding treatment options, particularly in cases where tumors are ER/PR negative. The fact that our study suggests the need for further confirmation through more definitive methods such as Fluorescence In-Situ Hybridization (FISH) or Chromogenic In Situ Hybridization (CISH) underscores the importance of accurate receptor status determination for effective therapeutic strategies.[17,18]

Prevalence of Equivocal Cases: The presence of equivocal cases is not uncommon, especially in populations with varying clinical practices and diagnostic criteria.[19] Our inability to confirm these cases through FISH may lead to an underestimation of true HER-2/neu positive cases in our study, impacting the perceived efficacy of therapies available for patients.

Limitations and Future Directions

The relative smaller sample size and also equivocal cases of Her2/neu were not confirmed by FISH due to financial constraints which has an effect of Her2/neu true positivity. These factors may affect the generalizability of the findings and the strength of associations drawn from the data. Future studies with larger cohorts and longer follow-up periods are necessary to confirm our findings and to explore the relationships between histological grade, receptor positivity, and clinical outcomes more comprehensively.

Additionally, the exploration of molecular subtypes of breast cancer in different populations may offer a more nuanced understanding of tumor biology and treatment responses. Collaborative efforts among researchers across Asia could facilitate multi-center studies that address the regional variability in breast cancer characteristics and improve outcomes for patients with hormone receptor-positive and HER-2/neu positive tumors [20].

CONCLUSION

In conclusion, our study contributes to the growing body of evidence regarding histological grading and receptor expression in breast cancer within Asian populations. The observed lower rates of ER and PR positivity, alongside the relatively high prevalence of Grade III tumors, highlight the need for ongoing research to understand the factors influencing these trends and to optimize treatment strategies tailored to specific patient demographics. Further investigations using advanced molecular techniques will be crucial in elucidating the complex biology of breast cancer and improving patient outcomes.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 08, 2024

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ISSN: 0975-3583,0976-2833 VOL15, ISSUE 08, 2024

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