

Immunomarker Study in Primary Breast Carcinoma: A 3 Year Cross Sectional Study in Rural Population of Western Odisha.

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ABSTRACT

Background: Breast cancer is the second most common cancer in the world and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012. It is well established that there are at least 4 main molecular subtypes of breast cancer based on different patterns of gene expression, and they have a considerable impact on outcome. Objective of this study is to establish a comparative analysis of breast cancer by IHC assay in western Odisha and to correlate with reference to tumor sizes, histological grade and lymph node involvement.

Methods: This study was conducted in VIMSAR, Burla, Odisha from July 2014 to August 2017 where 66 Cases of radical mastectomy received for routine histopathological evaluation from Dept of Surgery for histological typing and evaluation of IHC status by Dako Envision™ FLEX/HRP detection reagent. ER, PR, Her2neu and Ki67 were evaluated as per ASCO/CAP guidelines. The molecular subtypes were correlated with known prognostic markers as tumor size, histologic grade and lymph node status. **Results:** Triple negative and Her2neu 20.9% of cases were more aggressive and correlated with increased tumor size, higher grade and majority showed lymph node involvement at an early stage. **Conclusion:** After overall assessment it was seen that majority of cases were Luminal A type followed by triple negative and Her2neu positive cases indicating not only the latter are related to poor outcome but also showed its increased prevalence in this part of Odisha possibly because of late presentation. Molecular typing help categorize and plan targeted therapy to patients.

Keywords: Breast Carcinoma, Immunomarkers, Molecular Subtype, Nottingham Prognostic Index.

INTRODUCTION

Breast cancer today is the most common cancer in most cities in India, and 2nd most common in the rural areas. In India, the incidence of breast cancer has increased in the urban population, with 1 in every 22 women diagnosed with breast cancer.^[1,2]

As majority of people report to hospital only when they are symptomatic, therefore most patients are in stage 2B and beyond. Hence, these patients have lower survival rate. Cancers in the young are more aggressive. Many of these tumors are ER/PR negative, Her2 positive or triple negative and have worse prognosis than ER/PR positive tumors. An early detection using molecular subtypes guides effective targeted therapy thereby leading to better

prognosis. It is well established that there are at least 4 main molecular subtypes of breast cancer based on different pattern of gene expression, and they have a considerable impact on prognosis.^[3,4] Luminal A includes ER+ and/or PR+ and Her2-. Luminal A tend to have the best prognosis. Luminal B comprises ER+ and/or PR+ and HER2+, or ER+ and/or PR+ and Her2-tumors. The other 2 subtypes are the Her2neu over expressing tumors (ER-, PR-, Her2+) and the triple negative tumors (ER-, PR-, Her-), both of which confer bad prognosis.^[3] ER, PR, Her2neu and Ki67, routinely available in breast cancer specimens, are reliable, inexpensive simple, easy to interpret, reproducible, and readily available for clinicians without additional tests and useful for therapeutic decision making. The results of these tests are recorded in cancer registries allowing for population-based research which make them a reasonable substitute for the more expensive molecular subtyping.^[5,6] Our study is a cross sectional study done to establish a comparative analysis of breast cancer in rural part of western Odisha by Immunohistochemical(IHC) assay and to

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correlate it with tumor sizes, histological grade and lymph node involvement, which are already known prognostic factors to help clinicians determine its behavior

MATERIALS AND METHODS

This was a single centre cross sectional study over three year period (July 2014 to Aug 2017) at a tertiary care hospital. 66 Cases of radical mastectomy from all ages and both sexes were received for routine histopathologic (HP) evaluation from Dept of Surgery, VIMSAR, Odisha and were evaluated for HP typing and IHC status.

Exclusion Criteria

- Cases in which only a trucut biopsy or limited surgery was done.
- Cases having extensive tumor necrosis (without sufficient viable tumors cells).
- Her2neu (Equivocal cases).

Pathologic analysis

All the modified radical mastectomy specimens were inked and dimensions of tumors were taken. Using conventional H & E stain, the formalin fixed paraffin sections were stained and histopathologically examined. Histologic grading by Modified Bloom Richardson Grading (Nottingham Histologic Score) was given. IHC evaluation of ER, PR, & Her2neu of these cases comprising of Ductal Carcinoma in situ, invasive carcinoma and special type of carcinomas was done on 10% Neutral Buffered Formalin (NBF) fixed paraffin embedded tissue sections, using Dako FLEX Ready to use mouse monoclonal Antibodies (optimally diluted) and Dako Envision TM FLEX/HRP detection reagent.

For scoring ER and PR Allred score was considered, which included sum of proportion score and intensity score. Proportion score was calculated % positive cells as:

score 0 =0% tumor cells
score 1 = <1% tumor cells
score 2 = 1-10% tumor cells
score 3 = 11-33% tumor cells
score 4 = 34-66% tumor cells
score 5 = 67-100% tumor cells.

The intensity score for nuclear positivity of cells graded as:

score 0 as none
score 1 as weak
score 2 as moderate
score 3 as strong

Total Allred score of 0-2 considered negative and 3-8 was considered positive.

Her2neu was also reported with ASCO/CAP guidelines in which:

Score 0 (negative) no immunoreactivity or immunoreactivity in <10% tumor cells

Score 1+ (negative) faint weak immunoreactivity in >10% tumor cells and only portion of membrane positivity seen.

2+ (equivocal) weak to moderate complete membrane immunoreactivity in >10% of tumor cells or complete, intense, circumferential membrane staining in ≤10% of invasive tumor cells. Needs to be confirmed by FISH (florescent in situ hybridization).

3+ (positive) complete, crisp, intense, circumferential membrane staining in >10% of invasive tumour cells (chicken wire pattern).

ER, PR and Her2neu are categorised into molecular subtypes and correlated with Nottingham prognostic index.

Ki67 was also determined by counting at least 500 tumor cells regardless of the intensity in × 400 high-power field.^[5] The Ki-67 values were expressed as the percentage of positive cells in each case. Ki67 expression was categorized into 3 categories according to the score of Ki67 as:

Low, < 14% Ki67-positive cells

Intermediate, ≥ 14% and ≤ 30% Ki67-positive cells

High, > 30% Ki67-positive cells.^[6]

Ki 67 index was considered positive if 14% or more tumour cells were stained.

NPI: It is the most widely accepted Index, which uses 3 prognostic factor: lymph node stage, tumor size and histologic grade.

It is calculated using formula NPI= tumor size (cm) X 0.2+lymphnode (1-3) +grade (1-3).

Score <3.4- considered good prognosis.

Score 3.4-5.4 –moderate prognosis

Score >5.4-poor prognosis

Statistical analysis

We analysed the data using SPSS VERSION 22 SOFTWARE and applied Kruskal Wallis test to correlate NPI to molecular subtypes.

RESULTS

During the study period, Out of 66 cases, Infiltrating Ductal Carcinoma, Not otherwise specified (IDC,NOS) was the largest group, which accounted for 86.36% (57/66) of all cases and remaining were Medullary Carcinoma, Mucinous Carcinoma, 2 cases of Metaplastic Carcinoma (Squamous cell variant) and Mucinous Carcinoma with neuroendocrine differentiation. The median age of all cases was 49 years. According to TNM staging of Breast Cancer, majority of these tumors belonged to Stage IIB (T2N1M0). 36(54.54%) cases with tumor size between 2-5 cm (T2). [Table 1] 32(48.5%) cases were having N1 stage (≤3 lymph node involvement) with no clinical or radiographic evidence of distant metastasis(M0). [Table 2]. Among the total patients, 11 cases were grade I, 23 cases were grade II and 22 were grade III [Figure 1,1(a),1(b),1(c),1(d)]. Grade-II tumors were most frequently seen, followed by Grade-III tumors. [Table 3].

Table 1: Tumor Size.

SL NO.	Tumor Size in cm	Number of Patients	Percentages
1	T1(≤2)	00	00
2	T2 (>2,≤5)	36	54.54
3	T3(>5)	30	50
4	T4	00	00
	Total	66	100

Table 2: Lymphnode Status

SL NO.	Lymph Node Status	Number of Patients	Percentages
1	N0	29	43.94
2	N1	32	48.48
3	N2	05	7.57
4	N3	00	00
	Total	66	100

Table 3: Histological Grading.

SL NO.	Grading	Number of Patients	Percentages
1	Gr-I	11	16.67
2	Gr-II	23	34.85
3	Gr-III	22	33.33
	Total	66	100.00

Table 4: Association between Ki67 and histological grade

		Grade				Total
		No Grading	I	II	III	
Ki67	Negatie	5	10	9	1	25
	Positive	4	1	15	21	41
	Total	9	11	24	22	66

Table 4: Chi-Square Tests for

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	24.731a	3	.000
Likelihood Ratio	28.619	3	.000
Linear-by-Linear Association	16.388	1	.000
N of Valid Cases	66		

Table 5: Distribution of NPI across categories of molecular subtypes.

	Luminal A	Luminal B	Her2neu	Basal
NPI (<3.4) LOW	84.21%	50%	21.43%	-
NPI (3.4-5.4) MODERATE	15.78%	50%	64.28%	46.67%
NPI (>5.4) HIGH	-	-	14.28%	53.33%

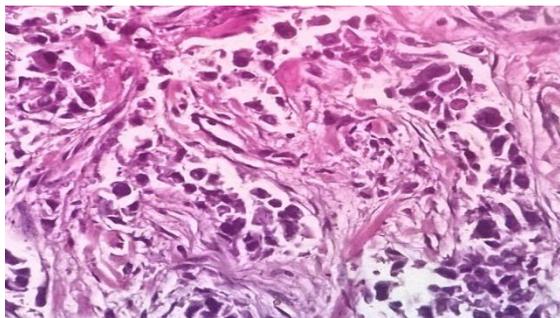


Figure 1: H&E 100x (Dyscohesive Cells with Vesicular Nucleus and Prominent Nucleoli)

Analyses showed that the grade III tumors demonstrated the highest frequency (21/22 cases of grade III; 95.45%) of Ki67 [Table 4] and of lymph node involvement, suggesting a significant relationship between tumor grade, lymph node status and Ki67 index (P=0.00).

Majority of Luminal A showed LOW NPI. Majority of Her2neu molecular subtype showed Moderate NP and majority of Basal subtype showed HIGH NPI. [Table 5] Distribution of NPI across categories of molecular subtypes were highly significant after applying kruskal wallis test which showed p=0.0001 (highly significant).

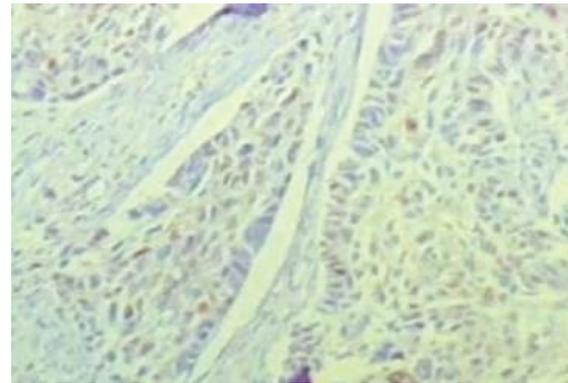


Figure 1(a): ER Negative 100x

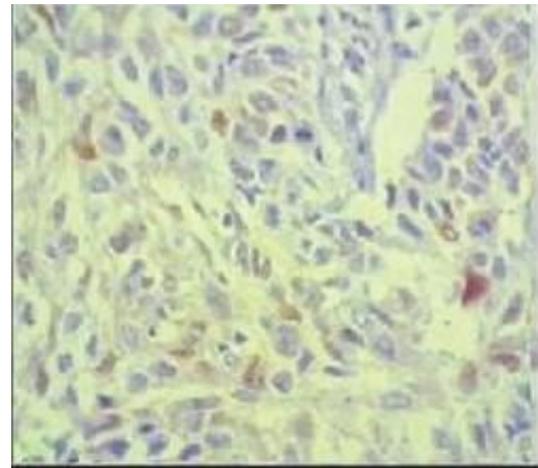


Figure 1(b): PR Negative 100x.

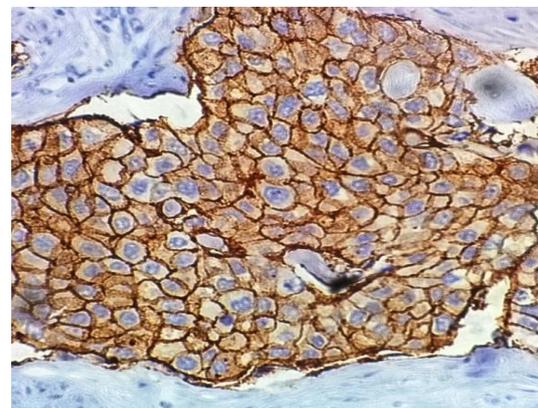


Figure 1(c): Her2neu positive (3+) 100x

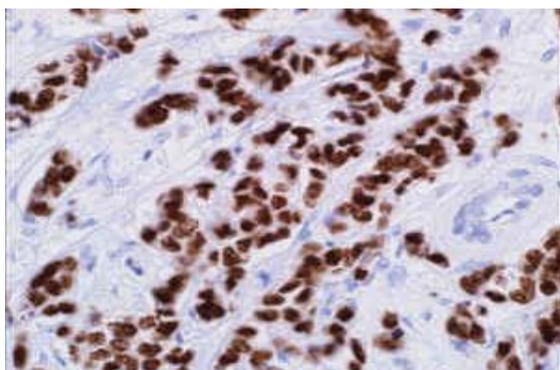


Figure 1(d): ki67 positive (70%)400x

DISCUSSION

Table 6: Association of ER, PR with HER2 status

Immunohistochemical subtypes	Onitilo AA et al(4) (%)	Huang JH et al(5) (%)	Present study (%)
Luminal A	68.9	66.4	34.85
Luminal B	10.2	30.9	6.06
TRIPLE negative	13.4	13.8	25.76
HER2neu positive	7.5	45.6	22.73

Present study data has less Luminal A type than other studies and more triple negative than rest of studies. Also Her2neu is higher than in the study done by Onitilo et al but lesser than the study done by Huang JH et al.^[7,8] In present study as patients presented late; Therefore, aggressive forms are more prevalent than found in other studies. [Table 6]

Table 7: Comparison of Ki67 (>=14%) with tumor grade in IDC (NOS)

Grade	Shokouh T et al(9) (%)	Madani et al(2016)(10) (%)	Present study (%)
I	6.7	19	09
II	56.4	62	65.21
III	36.9	26	95.45

Grade III tumor is more prevalent in the present study than other studies. [Table 7]

Table 8: Correlation of molecular subtypes with Ki67 (>=14%).

Molecular subtype	Shokouh T et al(9) (%)	Madani S et al(10)(2016) (%)	Present study (%)
Luminal A	15.2	45.9	13.04
Luminal B	51.2	39	60
Her2neu subtype	83.8	20.8	93.33
TRIPLE NEG	80.3	58.2	100

The present study showed a high Ki67 index in Her2neu and Triple negative cases, which well correlated with Shokouh T et al.[Table 8]

In a statistical study conducted at UK cancer registry, breast cancer has two age peaks (one was 50-59 years and other 65-70 years) but stabilized for

a period before increasing again from 75 years.^[11,12] It is seen that there is a rising trend of breast carcinoma in younger women especially in Asian countries.^[13] However in our study majority of cases (77.27%) were more than 40 years.

In present study incidence of tumors (>5cm) was 45.46% which was much higher than in other study such as by Raina V et al (2005).^[11]

It is also seen that Invasive ductal carcinoma and carcinoma in situ are the most prevalent type of breast carcinomas.^[7] In our study as well, invasive Ductal carcinoma was the most common carcinoma. Literature review revealed that Luminal A was more frequent subtype whereas the Her2 and triple negative were less.^[8,14] In the present study Luminal A subtype was the most prevalent followed by Her2neu and Triple negative subtype. Luminal B subtype was least.

In the present study, patients had greater HER2neu over expression compared with the patient reported in other studies.

Patients in present study had the highest frequency of HER2neu over expression related to invasive ductal carcinomas and the lowest frequency related to mucinous carcinomas, suggesting the higher invasiveness and greater aggressiveness of Ductal Carcinoma compared with other types. On the basis of this study, it appears likely that invasive ductal carcinomas have a greater likelihood of lymph node involvement compared with other types.

Medullary carcinoma had the highest frequency of Ki67 expression (90%) indicating a significant relationship between the type of tumor and Ki67 index.

Nottingham prognostic index (NPI) is a widely used integrated prognostic variable in patients with breast cancer. NPI has been correlated with tumor size, tumor grade, lymph node stage and patient survival. Ki67 was higher when NPI was high, indicating a poor prognosis. Similar results were also seen by A Kwatra et al.^[15]

CONCLUSION

Her2neu over expression and Basal subtype are more commonly seen in rural population of western Odisha, a possibility due to late presentation. It was seen that higher grade tumor had higher NPI values, Her2neu over expression, higher Ki67 index and a higher metastatic potential. It was seen that patients having Her2neu over expression responded to Herceptin (Transtuzumab) and lapatinib and not to Tamoxifen (selective ER modulator) therapy.^[16] A tumor expressing high Ki67 index shows better response to chemotherapy. Hence, routine assessment of ER, PR, Her2neu and Ki67 help guide treatment for patients and decide the treatment outcome effectively. It also prevents unnecessary treatment to patient such that it has minimum drug side effects and it encompasses less financial burden on patients.

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