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Status of ER,PR,HER2neu in Breast Carcinoma and its Relation to Histopathology in Determining The Survival Rate

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ABSTRACT

Aim : ER, PR and Her 2neu plays an important role in prognosis and determines the therapeutics in carcinoma breast. This study determines the hormone receptor status and its relation to histopathology.

Methods: This study was conducted at cancer hospital, Ranchi where one twenty eight case were assessed for the ER, PR, and Her 2neu status by immunohistochemistry along with histopathology in trucut biopsy as well as modified radical mastectomy specimen.

Results: 128 samples were studied for expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (Her 2neu). About 22.6 % cases had ER positive status while 19.5 % and 12.5 % were PR and Her2neu positive respectively. ER was negative in 10.1 % cases with PR negativity being 12.5 %. Her2 neu was negative in 17.1 % cases and equivocal (score 2) was seen in 5.4 % cases. Maximum number of patients were between the age group of 40-49 years. Lymph nodal involvement was seen in 31.2 % cases. 78.1 % cases had Modified Bloom Richardson grade III histologically of infiltrating duct carcinoma. (IDC)

Conclusion: Hormone studies helps to determine the overall disease along survival. Triple negative cases (ER/PR-, Her2-) had worst survival along with compared to the other subtypes. Histological subtypes and hormone status determines the prognostic and predictive value as well.

KEYWORDS : Estrogen, Progesterone , Her-2/neu receptor, Carcinoma breast, Histopathology

Introduction :

Carcinoma breast is on a rising trend and nearly one million women are affected worldwide every year.[1] In the year 2000 , 375,000 deaths were reported.[2] Research in the field of breast carcinoma is still ongoing for successful treatment outcome. Molecular classifications of breast carcinoma has been give emphasis upon , but still remains very expensive for general population. [3-11] Histopathological factors like tumour grade, size, lymph nodes and type determines the prognosis and response to therapy.

The ER, PR and Her2 neu status in breast carcinomas is practiced worldwide in higher centres. Tumours which are ER/PR positive have better prognosis than tumours which are not. ER strongly positive cases are benefitted from endocrine therapy alone, as compared to mild to moderate positivity. PR independently has disease-free and good survival.[12,13]

Her 2neu is also important predictive and prognostic factor for carcinoma breast. Her 2neu negativity is associated with chemotherapy resistance and aggressiveness. In this study the ER, PR, and Her 2neu status with correlation of histopathology helped to determine the survival rate and recurrence.

Methods and material:

This study was performed at RSJP Cancer Hospital in Ranchi, where patients presented to surgical oncology outdoor patient department (OPD) with complains of breast lump. Study period was of two years, from January 2014 to February 2016. The age of presentation, size of tumour, the nodal metastasis , metastases, skin and chest wall involvement along with prior chemotherapy status was recorded. Trucut biopsy was performed as OPD procedure done after taking consent of the patient. At least 5-6 core biopsy strips were taken and was sent in formalin. The biopsy was subjected to routine Haematoxylin and Eosin (H&E) staining following by ER,PR and He r2neu staining by immunohistochemistry (IHC). All equivocal cases of Her2neu were further tested and confirmed by Fluorescent In situ Hybridization (FISH) technique in these patient. While ER,PR and Her 2neu was also done on lumpectomy and modified radical mastectomy specimens (MRM).

Assessment by histopathology was done in all three kinds of speci-

men being trucut biopsy, lumpectomy and MRM specimens. Tumour grade was determined as grade I,II and III base on Bloom and Richardson Grading. Tumour size as well as nodal status were recorded. Post chemotherapy was recorded along with age which was divided as 20-29,30-39,40-49,50-59, 60-69 years and more than 70 years.

The ER and PR was scored from 0 and 8 depending on Allred score. Positive- Immunoreactive tumor cells present (≥1%) while negative - < 1% of immunoreactive cells, as shown in table 1

Table 1

Proportion Score	Positive Cells, %	Intensity	Intensity Score
0	0	None	0
1	<1	Weak	1
2	1-10	Intermediate	2
3	11-33	Strong	3
4	34-67		
5	>67		

Total score = Proportion Score + Intensity Score

While for Her 2 neu, score of 0,1,2 was given depending on immunhistochemistry profile.(Table 2)

Table 2

Result	Criteria
Negative (Score 0)	No staining observed or Incomplete, faint/barely perceptible membrane staining in ≤10% of invasive tumour cells

Negative (Score 1+)	Incomplete, faint/barely perceptible membrane staining in >10% of invasive tumour
Equivocal (Score 2+)	Incomplete and/or weak to moderate circumferential membrane staining in >10% of invasive tumour cells or Complete, intense, circumferential membrane staining in ≤10% of invasive tumour cells
Positive (Score 3+)	Complete, intense, circumferential membrane staining in >10% of invasive tumour cells

The IHC classification gene expression ER/PR+, Her2− with Luminal A ,ER/PR+, Her2+ with Luminal B; ER/PR−, Her2+ and ER/PR−, Her2− with triple negative/basal-like tumours.

Results :
The analysis of 128 patients was made from two years study period, January 2014 to February 2016. The maximum number of patients were in the age group of 40 to 49 years (45.31 %) followed by same percentage in the age group of 50-59 and 60-69 (18.75 % each)

Out of 128 patients with breast carcinoma, table 3 shows the grade, type and tumour size.

Table 3

Categorisation	No. of cases (%)
Tumour grade	
I	08(6.25 %)
II	20 (15.6 %)
III	100 (78.1 %)
Type	
Ductal	121 (94.5 %)
Lobular	05 (3.9 % %)
Other	02 (1.5%)
Tumour size	
< 2 cm	08 (6.25 %)
2-5 cm	117 (91.4 %)
>5 cm	03 (2.3 %)

The histological grade in most of the cases was III, as most patients did not present prior due to hesitancy and lack of knowledge.

Specimen received was trucut biopsy or modified radical mastectomy or lumpectomy . Trucut biopsy was performed in 50 subjects (39 %), MRM was done in 74 patients (57.8%) and lumpectomy was performed in only 04 cases (3.1 %) In this study ER was positive in 29 cases (22.6 %), PR was positive 25 cases (19.5%) while Her 2 neu was positive in 16 cases (12.5 %). Table 4 shows the ER/PR/Her 2neu dual positivity, negativity or triple positivity or negativity with lymph node involvement.

Table 4

|	ER.PR.Her2 neu +	ER.PR − Her 2 neu +	ER.PR −.Her 2 neu−	ER.PR/Her 2 neu -
No.(%)	70 (54.6%)	70 (59.3 %)	45 (35.1 %)	51 (39.3 %)
LN	17(13.2 %)	13 (10.1 %)	05 (7.8 %)	05 (7.8 %)
LN involvement	40 cases (31.2 %)			
LN uninvolved	88 cases (68.7 %)			

The overall survical and disease free survival rates are given in table no. 3.

The overall survival and disease free survival rates are given in table no.5.

Table 5

Disease-free and overall survival by tumour subtype, ER/PR and Her2 status		
Subtype	Disease-free survival	Overall survival
1. ER/PR status		
ER −	87.5 %	91.2 %
ER -	71.1 %	78.2 %
2. Her 2neu status		
Her 2 neu +	76.2 %	85.0 %
Her 2 neu -	83.2 %	87.1 %
3. Subtypes		
ER/PR/Her 2 neu +	89.2 %	85. 5 %
ER/PR/Her 2 neu -	78.3 %	71.8 %
ER/PR −/ Her 2 neu -	90.1 %	85.4 %
ER/PR - / Her 2 neu +	77.7 %	65.5 %

Among the 128 cases, 16 of the cases (12.5 %) were given neoadjuvant chemotherapy (NACT) following which MRM was done. In about 2 cases (1.5 %) histological assessment showed no residual viable tumour in post NACT cases. Following MRM and recurrence was seen in the form of chest wall nodule in 01 case (0.7 %), liver metastasis was seen in 03 cases (2.3 %), brain metastasis and bone metastasis was in 02 cases each (1.5 % each).
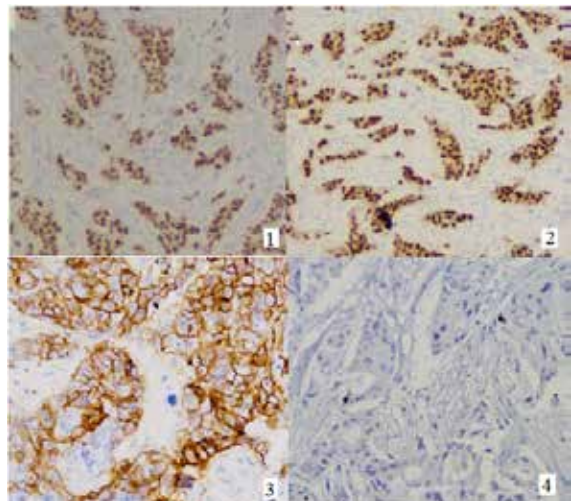
Fig .1 is ER positive, Fig. 2 is PR positive, Fig. 3 is Her 2neu positive while Fig. 4 is triple negative.

Discussion:
ER, PR and Her2/neu studied have become important in management of breast carcinoma patients and has proved to be a prognostic marker in adjuvant hormone therapy [14,15] . The results of our study showed ER, PR, Her 2neu expression in association with various clinicopathological factors.

In our study, ER, PR and Her2/neu positive expression was 22.6%, 19.5 % and 12.5 % respectively which well correlates with different researches.[16,17] . In positive ER/PR cases, it was found that negative expression of Her2neu was higher than positive cases, which was in contract with other studies [18,19]. The positive expression in ER and PR was more than negative expression which was again in contrast with other studies[20]. In Slamon *et al.* studies it was reported that Her 2neu was positive in 20-30 % cases while in our study it was 12.5 %. positive expression of Her 2neu in contrast to our study positive expression of Her 2neu was found higher than negative expression[21]. Her 2neu equivocal cases which was 5.4 % in our studies was subjected to FISH studies. In our study which correlated with other studies, where there was inverse relationship of ER and PR expression with Her 2neu [22,23].

Positive ER, PR with Her2 negative expression have better prognostic and therapeutic outcome that ER,PR negative with Her 2neu

positivity and triple negative ones. ER/PR positive expression, Her2 positive subtype varies with chemotherapy while and Her2 expression shows better chemotherapy response. Triple positive cases have recurrence rate high.[24] Retrospective study showed that ER/PR positive and Her 2neu cases have benefitted less from Tamoxifen.[25]

Our study had similar findings as other where triple negative and Her positive /EP negative cases where there was poor pathological and molecular prognosis.[26,27]. The triple negative cases had worst disease-free and overall survival rate.

The grade III tumours were more in our study as compared to other studies which had grade II more.[17] ER, PR, Her 2neu as well as metastasis to lymph node has no significant association which matched with other studies.[28,29,30] Tokatli et al. study showed Her 2neu expression associated with increased positive lymph node metastasis status was similar to our study.[31]

In our study, maximum cases were of infiltrating duct carcinoma with less cases of lobular and others. Our as well as other studies did not show association of histopathological type and ER/ PR/ Her 2neu expression.[18] Tumour size did not show any significant association with expression of ER/PR/Her 2neu in contrast to other studies.[29,32] Metastasis was seen in six out of 128 cases following resection and were mostly triple negative or Her 2neu positive /ER negative.

Conclusion :

In our study subtype of triple negative cases (ER-/PR-, Her 2neu-) has the worst disease-free and overall survival compared to the different subtypes. Inverse correlation of ER, PR was seen with Her 2neu expression. Our studied shows infiltrating duct carcinoma of grade III was found the most with tumour size of 2-5 cm has poor survival rate. No association of lymph node involvement was seen with ER, PR, Her 2neu expressions.

References :

1. Bray F, Sankila R, Ferlay J, Parkin DM. Estimates of cancer incidence and mortality in Europe in 1995. Eur J Cancer 2002;38:99-166.

2. Colditz GA, Bernard A, Rosner WY, Chen MD, Holmes SE. Risk factors for breast cancer according to estrogen and progesterone receptor status. J Natl Cancer Inst 2004;96:218-28.

3. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006;295:2492–2502

4. Chang HY, Nuyten DS, Sneddon JB, Hastie T, Tibshirani R, Sørlie T, Dai H, He YD, van't Veer LJ, Bartelink H, van de Rijn M, Brown PO, van de Vijver MJ. Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. Proc Natl Acad Sci U S A2005;102:3738–3743.

5. Dolled-Filhart M, Rydén L, Cregger M, Jirström K, Harigopal M, Camp RL, Rimm DL. Classification of breast cancer using genetic algorithms and tissue microarrays. Clin Cancer Res 2006;12:6459–6468

6. Liu R, Wang X, Chen GY, Dalerba P, Gurney A, Hoey T, Sherlock G, Lewicki J, Shedden K, Clarke MF. The prognostic role of a gene signature from tumorigenic breast-cancer cells. N Engl J Med 2007;356:217–226.

7. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817–2826.

8. Sørlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418–8423.

9. Sørlie T, Perou CM, Fan C, Geisler S, Aas T, Nobel A, Anker G, Akslen LA, Botstein D, Børresen-Dale AL, Lønning PE. Gene expression profiles do not consistently predict the clinical treatment response in locally advanced breast cancer. Mol Cancer Ther 2006;5:2914–2918.

10. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999–2009.

11. Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, Talantov D, Timmermans M, Meijer-van Gelder ME, Yu J, Jatkoe T, Berns EM, Atkins D, Foekens JA. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 2005;365:671–679.

12. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998;11:155-68.

13. Bardou JV, Arpino G, Elledge RM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol 2003;21:1973–9.

14. Donegan WL. Tumor-related prognostic factors for breast cancer. Cancer J Clin 1997;47:28-51.

15. Bast RC, Ravdin P, Hayes DF, Bates S, Fritsche H, Jessup JM. 2000 update of recommendations for use of tumor markers in breast and colorectal cancer: clinical practice guidelines of American Society of Clinical Oncology. J Clin Oncol 2001;19:1865-78.

16. Lakmini KB. Quick score of hormone receptor status of breast carcinoma: Correlation with the other clinicopathological prognostic parameters. Indian J Pathol Bacteriol 2009;52:159-63.

17. Kaul R, Sharma J, Minhas SS, Mardi K. Hormone receptor status of breast cancer in the himalayan region of northern India. Indian J Surg 2011;73:9-12.

18. Kapicl IT, Ušaj KS, Panjković M, Ivanović DD, Golubović M. HER-2/neu overexpression in invasive ductal breast cancer–an association with other prognostic and predictive factors. Arch Oncol 2007;15:15-8.

19. Naeem M, Nasir A, Aman Z, Ahmad T, Samad A. Frequency of her-2/neu receptor positivity and its association with other features of breast cancer. J Ayub Med College Abbottabad 2008;20:23-6.

20. Janet L, Raymond S. Breast cancer incidence in young women by estrogen receptor status and race. Am J Public Health 1989;79:71-3

21. Slamon DJ, Clark GM. Amplification of C-ERB-B2 and aggressive breast tumors? Science 1988;240:1795-8.

22. Rosai J. Rosai and Ackerman's surgical pathology. 8th ed. Mosby, Edinburg; 1996. p. 1786-820.

23. Ayadi L, Khabir A, Amouri H, Karray S, Dammak A, Guermazi M, et al. Correlation of HER-2 over-expression with clinicopathological parameters in Tunisian breast carcinoma. World J Surgical Oncol 2008;6:112.

24. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, Ollila DW, Sartor CI, Graham ML, Perou CM. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007;13:2329–2334.

25. Fan C, Oh DS, Wessels L, Weigelt B, Nuyten DS, Nobel AB, van't Veer LJ, Perou CM. Concordance among gene-expression-based predictors for breast cancer. N Engl J Med 2006;355:560–569.

26. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006;295:2492–2502.

27. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, Ollila DW, Sartor CI, Graham ML, Perou CM. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007;13:2329–2334.

28. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res2007;13:4429–4434.

29. Ariga R, Zarif A, Korasick J, Reddy V, Siziopicou K, Gattuso P. Correlation of Her-2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. Breast J 2005;1:278-80.

30. Bozcuk H, Uslu G, Pestereli E, Samur M, Ozdogan M, Karaveli S. Predictor of distant metastasis at a presentation in breast cancer: a study also evaluating associations among common biological indicators. Breast Cancer Res Treat 2001;68:239-48

31. Tokatli F, Altaner S, Uzal C, Ture M, Kocak Z, Uygun K. Association of HER-2/neu overexpression with the number of involved axillary lymph nodes in hormone receptor positive breast cancer patients. Exp Oncol 2005;27:145-9.

32. Prati R, Apple SK, He J, Gornbei JA, Chanh HR. Histopathologic characteristics are predicting HER-2/neu amplification in breast cancer. Breast J 2005;11:433-9.