Section: Pathology

Original Research Article

STUDY OF P53 EXPRESSION, ITS CORRELATION WITH ER, PR AND HER-2NEU STATUS IN CARCINOMA BREAST

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ABSTRACT

Background: Breast cancer being the most common cancer among women in India and other regions of the world, constant research on prognostic and predictive markers of breast carcinoma are going on. Early diagnosis and appropriate treatment can prolong the survival of patients diagnosed with breast cancer. **Objective:** To assess the ER/PR status of breast carcinoma and to assess the HER-2 neu status of breast carcinoma,

Materials and Methods: This prospective study was undertaken over a period of 2 years from October 2018 to September 2020 in the Department of Pathology at Chalmeda Anand Rao Institute of Medical Sciences.

Result: 27 cases (54%) which were ER and PR positive showed p53 negative in all cases. 9 cases which showed P53 positivity were ER and PR negative. ER & PR correlation with p53 positivity was found to be statistically significant 0.001 ($p < 0.01$). 18 cases which were HER-2neu positive showed p53 negative. 9 out of 32 cases with HER-2neu negative showed p53 positivity. HER-2neu correlation with p53 positivity was found to be statistically not significant 0.04 ($p > 0.01$). ER & PR correlation with p53 positivity was found to be statistically significant 0.0001 ($p < 0.01$) whereas other prognostic variables were not statistically significant.

Conclusion: In the present study of ER, PR, Her-2neu and p53 expression in breast carcinoma by IHC method indicates higher rates of positive expression with various clinicopathological aspects. Higher number of grade-I tumours showed ER, PR positivity as compared to grade III tumours. Inverse relationship was observed between Her-2neu and p53 with that of ER and PR.

Keywords: p53 expression, ER,PR, HER-2neu status, Carcinoma Breast.

INTRODUCTION

Breast cancer is the most common cancer in the world in developed countries and 12% of breast cancer occur in women between 20-34 years.[1] India accounts for nearly six percent of deaths due to breast cancers in the world and also one out of every 22 women in India is diagnosed with breast cancer every year.[2]

In India, premenopausal women constitutes about 50% of all breast cancer patients. Breast cancer risk in India revealed that lifetime duration of breast feeding was inversely associated with breast cancer risk among premenopausal women.[3]

Several histopathological features have prognostic significance in breast carcinoma which includes histologic subtype, grade, lymph node status, ER/PR status, HER-2 neu, growth factor and its receptors, Ki-67, oncogenes and tumor suppressor genes(p53).[4]

In recent years, for therapeutic and prognostic purposes breast cancer once diagnosed, is then subjected to immune-histochemical studies (IHC) which commonly include estrogen receptor (ER) and progesterone receptor (PR). With advent of molecular classification of breast carcinoma along with these, other ancillary cytokeratin prognostic markers (BRCA1, BRCA 2, p53, Bcl 2 and Ki 67)

have markedly revolutionized the research for breast cancer.[5]

Immunohistochemistry (IHC) refers to the process of detecting antigens in cells of a tissue section by exploiting the principles of antibodies binding specifically to antigens in biological tissues.[6] IHC is the common available method used to detect abnormal cellular phenomena (including malignant tumors, aberrant proliferation or cell death), localize the distribution of different biomarkers in tissues and determine biomarker status.[7]

Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2/neu) are routinely done in breast carcinoma. It not only helps in the prognosis of the tumor but also helps in deciding its treatment. The goal of doing this receptor status is to provide right treatment to the right patient.[8]

p53 is the main regulator of genomic stability through regulation of the cell cycle. Over expression of p53, which is caused by TP 53 mutation, is the most frequent genetic alteration in not only breast cancers but also in various malignancies such as ovarian, esophageal and GIT.[9]

Breast tumors expressing a high amount of p53 are more frequently ER- negative and PR-negative and are also associated with a high proliferation rate, high histological and nuclear grades, aneuploidy and poorer survival.[10]

The present study was done with the objective to find expression and correlation of ER, PR and p53 in breast carcinoma as well as to correlate expression of these tumor markers with histological type, grade and other parameters in a small cohort of Indian female population suffering from breast cancer.

MATERIALS AND METHODS

This is a prospective study with histologically proven diagnosis of breast carcinomas in the department of pathology at Chalmeda Anand Rao Institute of Medical Sciences during the period of October 2018 to September 2020.

Inclusion Criteria: Mastectomy specimens, lumpectomy and trucut biopsies from breast carcinoma in female patients of reproductive and postmenopausal ages were included in the study.

Exclusion Criteria

Cases where there was extensive tumor necrosis without sufficient viable tumor cells for accurate evaluation of the immunohistochemical results.

All the post chemotherapy and male breast cases.

Non-epithelial malignancies.

The detailed clinical history and results of relevant investigations done were collected from the patient's case files. The mastectomy and lymph node dissection specimen were received in the pathology department in 10% neutral buffered formalin. In every case the standard protocol for surgical grossing of radical mastectomy specimens was followed. Specimens was kept for fixation for 24hrs.

After a detailed specimen description, multiple sections were taken from the tumour, surgical margins, nipple and areola, non-neoplastic breast all lymph nodes. After conventional processing and embedding in paraffin wax, sections of 4-5 micrometre were cut using rotor microtome and stained using hematoxylin and eosin (H&E) for histopathological study

Immunohistochemistry procedure

1. The poly-L-Lysine coated representative sections were labelled appropriately (i.e. biopsy number and antibody name) with diamond pencil.
2. Sections were placed on hot plate at 500c for 15 minutes on till wax is dissolved and then successively passed through following solutions for deparaffinisation.

and rehydration. Xylene I	**10min**
Xylene II	10min
100% alcohol	5min
90% alcohol	5min
70% alcohol	5min
60% alcohol	5min

3. Sections were washed in tap water 1-2 min each.
4. Antigen retrieval was done with citrate buffer (pH-6) or Tris EDTA buffer(pH-9) using biogenex EZR microwave oven at 950C for two cycles for 13minutes each. Slides were brought to room temperature and washed with distilled water.
5. Slides were then treated with endogenous peroxidase block for 10minutes.
6. Further slides were washed in wash buffer (phosphate), 3 times for 3 minutes.
7. Treated with power block for 10minutes, the solution was allowed to drain.
8. Primary antibody was applied for an hour.
9. Washed with wash buffer, 3 times for 3 minutes.
10. Super enhancer was added for 20 minutes.
11. Secondary antibody was then added to the sections and incubated for 30min in a humid chamber. The slides were washed in TRIS buffer 3 times, 3 min each.
12. DAB solution was prepared freshly and added to sections for 8-10min and monitored microscopically for the development of colour.
13. The sections were washed in distilled water and counter staining was done with Harri's hematoxylin for 45sec. Blueing was done by washing the sections in running tap water.
14. Sections were dehydrated using ascending grades of alcohol, cleared in xylene and mounted with DPX.

Each batch should contain a positive control i.e, where staining state is known and a negative control i.e to pick up the background staining. The sections of negative control are incubated with TRIS buffer instead of primary antibody.

Immunohistochemical scoring system for ER, PR and Her neu.[11] Allred system of scoring for ER and PR:

ER and PR are nuclear receptors. In the all red system of scoring, score 0-5 is given to the cells depending on the proportion of cells which are stained(proportion score-PS) and score 0-3 is given depending on the intensity of staining (intensity score- IS). By adding both PS and IS we can calculate final Allred score (PS+IS=AS).

Scoring for Human epidermal growth factor receptor 2neu overexpression:

Her2neu is a cell membrane receptor and depending on the intensity of staining a score 0-3 is given to the cells.

RESULTS

In the present study, age ranged from 31-80 years and the mean age+ SD was Majority, 17 cases belonged to 41-50 years followed by 12 (51-60), 11 (61-70),8(31- 40) and 2 cases of age 71-80.

In our study, all the cases presented with breast lump which was the commonest symptom in 39 cases(78%), followed by breast lump with pain in 8 cases(16%), breast lump with discharge in 2 cases(4%) and 1 case had breast lump with ulcer(1%).

In our study, majority of cases 27(54%) presented within 4-6months, followed by 12 cases (24%) 7-9 months, 6 cases (12%) 10-12 months and 5 cases (10%) within 3 months.

Majority of cases 29(58%) presented on the left side and 21 cases (42%) presented on the right side. None had bilateral involvement.

Table 1: Location of tumor

QUADRANT	NO. OF CASES	PERCENTAGE
Upper outer	28	56%
Upper inner	11	22%
Lower outer	8	16%
Lower inner	2	4%
central	1	2%

Majority 28 cases (56%) showed tumor in upper outer quadrant, followed by 11 cases (22%) upper inner, 8 cases (16%) lower outer, 2 cases (4%) lower inner and 1 case (2%) was central.

In our study, majority 39 specimens (78%) were modified radical mastectomy and 11 (22%) were biopsies

On gross examination, 21 cases (42%) measured between 2.0-5.0cms, followed by 18 cases (36%) >5cm and 11 cases (22%) <2.0cm.

Histologic grading showed 25 cases (50%) to be grade I, followed by 14 cases (28%) to be grade II and 3 cases (6%) to be grade III.

Table 2: Histopathological diagnosis

Diagnosis	Number of patients	Percentage
IDC-NOS	42	84
Papillary carcinoma	04	8
Mucinous carcinoma	02	4
Lobular carcinoma	02	4

In our study the predominant histologic subtype was infiltrating ductal carcinoma (NOS)- 42cases (84%), 4 cases (8%) were papillary carcinoma, 2 cases (4%) each of mucinous carcinoma and lobular carcinoma.

29 cases (58%) were reactive, and 10 cases (20%) had nodal metastasis. In cases of biopsies lymph nodes were not available for study.

Table3: Correlation of p53 with ER, PR and HER-2 status

ER, PR, HER-2	Number patients	%	p53 positive	p53 negative	P- value
ER					
Positive	27	54	-	27	0.0001 $p < 0.01^{**}$
Negative	23	46	9	14	
PR					
Positive	27	54	-	27	0.0001 $p < 0.01^{**}$
Negative	23	46	9	14	
HER-2					
Positive	18	36	-	18	0.04 $p > 0.01$
Negative	32	64	9	23	

In our study, 27 cases (54%) expressed ER and PR, 18 cases (36%) expressed HER-2 positive. P53 was expressed in 9 cases which were triple positive. ER & PR correlation with p53 positivity was found to be statistically significant ($p < 0.01$) whereas her-2neu correlation with p53 positivity was not significant ($p > 0.01$).

Table 4: Relationship between histologic subtypes and ER, PR and Her-2 positivity

Histologic subtype	ER+ (n=27)	PR+(n=27)	HER-2 +(n=18)
IDC(NOS)	20 (74%)	20 (74%)	17 (95%)
Lobular carcinoma	1 (2%)	1 (2%)	0 (0%)
Papillary carcinoma	4 (15%)	4 (15%)	1 (5%)
Mucinous carcinoma	2 (9%)	2 (9%)	0 (0%)

In our study, the most common histological type in which ER, PR and HER-2 positivity were noted was IDC(NOS). 20/27(74%) of ER positive cases, 20/27 cases (74%) of PR positive cases and 17/18(95%) of HER- 2 positive cases were IDC(NOS). there was 1 case of lobular carcinoma positive for both ER and PR.

4 cases of papillary carcinoma positive were for both ER and PR and 1 case were positive for HER-2. 2 cases of mucinous carcinoma were positive for both ER and PR.

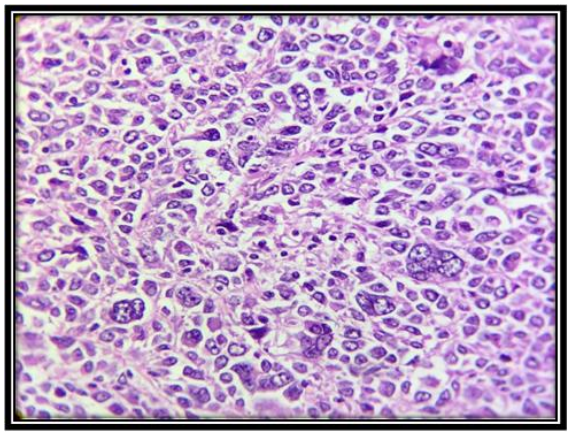

Figure 1: High power view of grade III IDC-NOS

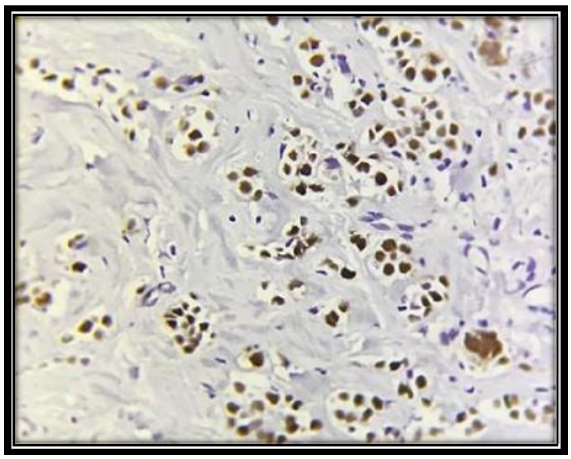

Figure 2: Grade III IDC showing P53 positivity (40x)

p53 expression in relation to hormonal status: 27 cases which were ER and PR positive showed p53 negative in all cases. 9 cases which showed P53 positivity were ER and PR negative.

p53 expression in relation to HER-2 neu: 18 cases which were HER-2 neu positive showed p53 negative. 9 out of 32 cases with HER-2 neu negative showed p53 positivity.

ER & PR correlation with p53 positivity was found to be statistically significant 0.0001 ($p < 0.01$) whereas other prognostic variables were not statistically significant. [Table 5]

Table 5: Table 5: p53 expression in relation to hormonal status, her-2 and other conventional prognostic variables of breast carcinoma

Variable	p53 +	%	p53 -	%	Total	%	P-value
1.ER							
• ER +ve	0	0	27	100	27	54	0.0001** p <0.01
• ER –ve	9	40	14	60	23	46	
2.PR							
• PR +ve	0	0	27	100	27	54	0.0001**
• PR –ve	9	40	14	60	23	46	p <0.01
3.HER-2							
• HER-2 +ve	0	0	18	100	18	36	0.042
• HER-2 -ve	9	28	23	72	32	64	p >0.01

DISCUSSION

The most common age group affected in our study was 41-50 years which was in concordance with Bhagat vasudha M et al.[1] The age range affected in the study done by sanjay piplani et al,[12] was 50-60 years, in the study done by Cherry Bansal et al,[13] it was 55-65 years and it was between 50-70 years in the study by Alireza Abdollahi et al,[14] the age group affected was 45-55 years in a study done by Pathak TB et al.[15]

In the present study, 28 cases (56%) had tumor in upper outer quadrant. Similar observation was made by Meena et al,[16] (54%) and Costa M et al 17(54.1%). The tumors in the lower outer quadrant was (16%) which was similar to Costa m et al. The tumors in lower inner quadrant were 4% which was in concordance with Costa et al (4.7%).

In the present study 21 cases(42%) tumors were between 2-5 cm. in Bhagat vasudha et al,[1] study, 38 cases (65.5%) were between 2.5cm, it was 44 cases (67.5) in Sanjay piplani et al,[12] and 74 cases (69.8) in Ahmed et al,[18] study.

The present study 42 cases (84%) were Invasive ductal carcinoma-NOS. similar observation was made by Robab sheikhpour et al,[19] Bhagat Vasudha M et al,[1] and Peiro et al.[20] The present study showed 2 cases (4%) of infiltrating lobular carcinoma which was in concordance with Bhagat Vasudha M et al 2 cases (3.44%). Other types of carcinoma had varied incidence in different studies.

In the present study, histological grading was done using modified bloom Richardson grading, majority 59.5% were grade I and 33.3% were grade II which was in concordance with Sanjay piplani et al,[12] grade III tumors were variable in different studies.

In the present study, only 10 cases (20%) showed lymphnode metastasis, 29 cases (58%) showed reactive lymphnodes and for 11 cases (22%) lymphnodes were not available. In the study done by Sanjay Piplani et al 64.6% cases showed positive lymphnodes, whereas it was 53.4% in Bhagat Vasudha et al and 75.3% Hussain gadelkarim et al.

CONCLUSION

In the present study of ER, PR, Her-2neu and p53 expression in breast carcinoma by IHC method indicates higher rates of positive expression with various clinicopathological aspects. Higher number of grade-I tumours showed ER, PR positivity as compared to grade III tumours. Inverse relationship was observed between Her-2neu and p53 with that of ER and PR. As the tumor grade increases, ER and PR expression decreases and Her-2neu and p53 expression increases.

REFERENCES

1. Bhagat Vasudha M,Jha Bharti M,Patel Prashanth R: Correlation Of Hormonal Receptor And Her-2/Neu Expression In Breast Cancer : A Study At Tertiary Care Hospital In South Gujarat,Vol 2, July-Spetember 2015.
2. Manuneethimaran Thiygarajan*, Nitesh Navrathan, Mohanapriya T., Arun Kumar, Balaji Singh: Correlation between estrogen receptor, progesterone receptor, HER2/neu status and other prognostic factors in carcinoma breast in Indian population, International Surgery Journal , October-December 2015 | Vol 2.
3. Meshram II, Hiwarkar PA, Kulkarni PN. Reproductive risk factors for breast cancer: a case control study. Online J Health Allied Scs. 2009;8(3):5.
4. Mohammad naeem, amir nasir, zahid aman, tariq ahmad, ambreen samad. Frequency of her-2/neu receptor positivity and its association with other features of breast cancer. J Ayub med coll Abbottabad,2008;20(3):23-26.
5. Jeffery SR. Predictive and prognostic molecular markers in breast cancer. In: Lowe DG, Underwood JCE, editors. Recent Advances in Histopathology. London: Royal Society of Medicine Press Ltd; 2005. p. 31-50.
6. Omranipour R, Fattahi A. Prevalence of ductal carcinoma insitu of the breast in Tehran University Medical Centers: evaluation of 2244 cases. Tehran Univ Med J 2009;67(7):516-21
7. Azizun – Nisa, Bhurgri Y, Reza F, Kayani N. comparison of ER, PR and HER-2 reactiving pattern with histologic grade, Tumor size and lymphocytic status in breast cancer. Asian pac J Cancer Prev 2008;9(4):553-6.
8. Banyameen Mohamad Iqbal, Archana Buch: Hormone receptor (ER, PR, HER2/neu) status and proliferation index marker (Ki-67) in breast cancers: Their onco-pathological correlation, shortcomings and future trends, 2016 Medical Journal of Dr.D.Y.Patil University, Published by Wolters Kluwer – Medknow.;vol- 9,issue-6.
9. Lee SK, Bae SY, Lee JH, et al. Distinguishing LowRisk Luminal A Breast Cancer Subtypes with Ki-67 and p53 Is More Predictive of Long-Term Survival. PLoS One. 2015;10(8):e0124658.
10. Lacroix M, Toillon RA, Leclercq G. p53 and breast cancer, an update. Endocr Relat Cancer. 2006;13(2):293-325.
11. MOLL U. and SCHRAMM L.: P53 an acrobat in tumorigenesis. Crit. Rev. Oral. Biol. Med., 9: 23-37, 1998.
12. Rosenblum MG,Horn SA & Cheung Lh. A novel recombinant fusion toxin targeting her-2neu overexpressing cells and containing human tumor necrosis factor. Int J cancer 2000;88(2):267-73.
13. HOOPER M.: The role of p53 and Rb-1 genes in cancer, development and apoptosis. J. Cell. Sci., 18: 13-17, 1994.
14. MOLL U. and SCHRAMM L.: P53 an acrobat in tumorigenesis. Crit. Rev. Oral. Biol. Med., 9: 23-37, 1998.
15. PRUSINER S.B.: Molecular genetics of transmissible spongiform encephalopathies. Proc. Natl. Acad. Sci., 95: 13363-13383, 1998.
16. LACROIX M., TOILLON R.A. and LECLERCQ G.: p53 and breast cancer, an update. Endocr. Relat. Cancer, 13: 293-325, 2006.
17. Ahmed HG, Al-Adhraei MA, Al-Thobhani AK. Correlations of Hormone Receptors (ER and PR), Her2/neu and p53 Expression in Breast Ductal Carcinoma Among Yemeni Women.The Open Cancer Immunology Journal. 2011;4:1-9.
18. Lal P, Tan Kl, Chen B; Correlation Of Her-2 neu status with estrogen and progesterone receptors and histologic features in 3655 Invasive Breast Carcinomas. American journal of clinical pathology, 2005; 123(4): 541-546.
19. Chandrika rao, Jayaprakash Shetty, Hl Kishan Prasad. Morphological profile and receptor status in breast carcinoma. An institutional study. Journal of cancer research and therapeutics 2013;9(1):44-49.
20. Pawan Nikhra, Smita Patel, Dilip Taviad and Shalu Chaudhary. Study of ER (Estrogen Receptor), PR (Progesterone Receptor) & HER2/NEU (Human Robab Sheikhpour, Fatemeh Poorhosseini. Relation between Estrogen and Progesterone Receptor Status with p53, Ki67 and Her-2 Markers in Patients with Breast Cancer,Iranian Journal Of Blood And Cancer, Volume 8,Issue 4 December 2016.
21. Peiro G, Adrover E, Aranda IF, Peiro Mf, Neivero M, Sanchez Paya J. Prognostic implications of her-2 status in steroid receptor positive, lymph node negative breast carcinoma ,Am J Clin Pathol,2007;127:780-86.
22. Onitilo AA, Engel MI, Greenlee TR, Mukesh NB. Breast cancer subtypes based on ER/PR and HER-2 expression: comparision of clinicopathological features and survival. Clinical medicine and research;7(1/2):4-13.
23. Chandrika rao, Jayaprakash Shetty, Hl Kishan Prasad. Morphological profile and receptor status in breast carcinoma. An institutional study. Journal of cancer research and therapeutics 2013;9(1):44-49.