



ORIGINAL RESEARCH PAPER

Pathology

IMMUNOHISTOCHEMICAL PROFILE OF INVASIVE BREAST CARCINOMA PATIENTS: A STUDY AT TERTIARY CARE HOSPITAL IN CENTRAL INDIA

KEY WORDS: Breast carcinoma, CK5/6 receptor, Estrogen Receptor, Her-2/neu receptor, Immuno-histochemistry, Progesterone Receptor, Ki-67

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ABSTRACT

Background: Breast carcinoma is now becoming a commoner type of cancer following cervical cancer. Hormone receptors analysis is very useful for molecular profiling, to ascertain the correct treatment and for further outcome of the patient. The objective of study was to explore the positivity of Estrogen, Progesterone, Her-2/neu, Ki-67 and CK 5/6 receptor among breast carcinoma cases and to express its association with clinic-pathological condition of patients.

Methods and Material: This study was conducted in department of pathology government medical college. Study duration was 2 years. Patients who had invasive breast carcinoma were included in study. Sample size of study was 70 patients. Immunohistochemistry for ER, PR, Her-2/neu, Ki-67 and CK5/6 expression was done in all cases. Chi square test was applied to find out association between various hormonal markers.

Result: In this study, ER expression was seen in 51.4%, PR in 47.1% of cases and Her2/neu positivity seen in 51.4%. Expression of Ki-67 and CK 5/6 was seen in 22.9% and 21.4% of cases respectively. Strong correlation was observed in between hormone receptor expression and Her-2/neu, Ki-67 and CK5/6 positivity.

Conclusion: ER, PR, Her-2/neu, Ki-67 and CK5/6 positivity were well correlated with patient Clinicopathological condition of patients. Expression of ER, PR were associated with small tumor size, less number of lymph node involved and grade I & II of breast cancer whereas expression of Her-2/neu, Ki-67 and CK5/6 were seen in tumor having large size, more number of lymph node involved and grade II & III of breast cancer.

Introduction:

Cancer is rapidly increasing in all parts of world and causing mortality in all age groups. Breast cancer is one of them that became leading cause of death among women globally.¹ In India breast cancer is coming next to cervical cancer in term of incidence and mortality.² Its incidence is relatively low as compare to western provinces but it's rapid rise is expected in coming years.³ Breast cancer treatment experienced a drastic change in last few years. It changed toward reconstructive and conservation from unfaster way.⁴ There is several prognostic factor available for breast cancer that give us the information of patient survival and risk of recurrence like tumor size, histological type, grade and number of auxiliary lymph node involved.⁵ Now a days, Hormone receptors like estrogen receptors (ER), progesterone receptors (PR), her-2/neu analysis is very useful for molecular profiling, to ascertain the correct treatment and for further outcome of the patient.³ Based on this background present study aimed to find out whether the hormone receptor expression co-inside with patient clinical and histopathological profile.

Material and Method:

This study was conducted in department of pathology government medical college. Study duration was 2 years from 2012 to 2014. Patients who had invasive breast carcinoma were included in study. Sample size of study was 70 patients.

The immuno staining starts with cutting of formalin – fixed, paraffin embedded tumor section that have thickness of 3-4 mm. these cut sections were put on hot water surface having 45oC temperature. Then this section was spread wrinkle freely on poly L lysine coated slide. This slide was deparafinized in xylene solution. After deparafinization rehydration process were started through serial levels of alcohol. Finally slides were placed in phosphate buffer. Antigen was retrieved in pressure cooker by citrate buffer. Then peroxide solution was used to block endogenous peroxide activity. Antigen detection was done using Monoclonal antibodies related to various hormone receptors then added polymer horse radish peroxidase. 3, 3'- diaminobenzidine tetrahydrochloride (DAB) was added for colour production. Positive and negative control for each marker was stained for every IHC staining batch.

These slides were screened manually for hormone receptor positivity. As per the finding of Previous study cut off was set to be 10% neoplastic cells.^{3,4,6,7} The positive control was known ER/PR

positive cancer cases. Her-2/neu receptor expression was scored based on previous studies.^{4,6} A score of 3+ considered positive for receptor. The positive control for Her-2/neu was known cases and internal control was negative benign condition. In this study if Slide showed more than fourteen percent neoplastic cell nuclear immune-reactivity it considered to be Ki- 67 receptor positive. The germinal center of lymph node was positive control. Positive for Cytokeratin 5/6 was considered if 11-100% tumor cells showing Cytoplasmic immunoreactivity. The skin epidermal layer was positive control for ck 5/6. This study was approved was institutional review board.

Data was entered in Microsoft office excel 2007 and Chi square test was applied using SPSS version 20. Phi Contingency coefficient was calculated to find out association. P value found below 0.05 considered to be statistically significant.

Result:

These 70 patients were belonging to age group ranged from 25 to 70 years. Majority of tumors were located in the left breast (68.6%) and in upper outer quadrant (57.1%). ER expression was seen in 51.4%, PR in 47.1% of cases and Her2/neu positivity seen in 51.4%. Expression of Ki-67 and CK 5/6 was seen in 22.9% and 21.4% cases respectively.

In present study Hormone receptors were found as follows: ER+/PR+ in 32(45.71%) cases, ER+/PR- in 4(5.71%) cases, ER-/PR+ in 1(1.43%) and ER-/PR- in 33(47.14%) cases. This study showed that ER and PR positivity were strongly associated with phi contingency coefficient 0.861 and p value<0.001. When we compared the association between ER, PR and Her-2/neu it was found to be inversely associated with phi contingency coefficient 0.029 and 0.056. Cytokeratine receptor 5/6 positivity was inversely associated with other receptors in study with negative coefficient value.(Table 1)

It was also observed that ER and PR positivity was increasing with increasing age of patients. Expression of ER and PR were decreasing as the size of tumor advances, whereas expression of Her-2/neu and CK5/6 were increasing with advancing tumor size. Hormone receptor positivity and lymph node involvement was inversely associated. Although expression of CK5/6 was seen in cases with more number of lymph node involved. (Table 2)

Expression of ER and PR were seen more in cases had grade I and II of Bloom Richardson grading whereas Her-2/neu positivity, high Ki-67 and CK 5/6 positivity more associated with grade II and III of BR grading. (Table 2)

Discussion:

This study comprised of cases of infiltrating breast carcinoma with mean age of 47.8 ± 11.1 years. In India mean age of patients with breast cancer lower as compared to western countries. This may be due to lifestyle related factors such as advanced age of marriage, westernization of diet and reduced feeding through breast which was associated with breast cancer occurrence in comparatively young age group.^{3,9} In this study most of the cases had breast carcinoma in left breast (68.6%) and in upper outer quadrant (57.1%). This was similar to study of Ambrose M et. al (2011)³.

In this study, Most of the cases could not developed lymph node metastasis but other studies which was carried out in different part of India showed higher percentage of breast carcinoma with lymphnode metastasis this was much lesser than studies of western world.^{7,8} Tumor size in present study was found to be more than 2 centimeter in 98.5% of cases. This was similar to various studies.^{1,7,9} Despite to this, studies conducted in western countries found that tumors are predominantly had tumor size less than 2cm.10 This is because of the early screening programs prevalent in western world.

In present study it observed that, ER was positive in 36 cases (51.4%) and PR in 33 cases (47.1%). The study done by Shet T. et al (2009)¹⁴ showed that expression of hormonal receptors were decreased over the years.

The Following table shows comparative results of hormonal receptor in this study with various other studies done on different parts of world. (Table 3 and 4)

The percentage of tumors expressing PR but not ER was 1.43% in our study. The study done by Ambrose M et al (2011)³ found figure of 4.05% and study done by shet T et al (2009)¹⁴ and Rhodes A et al (2000)¹¹ found 3.4% and 3.0% respectively.

There was strong association found in between ER expression and PR expression. An inverse relation was found in between Her-2/neu and ER and PR expression. This was similar to various studied done in different parts of world.^{6,7,9,10,15} ER, PR, Her2/neu and CK 5/6 expression are positively correlated with Ki-67. An inverse correlation was found in between CK 5/6 expression and ER, PR and Her-2/neu expression, however CK 5/6 expression was positively correlated with Ki-67 expression and this finding was statistically significant.

There was no significant correlation was observed between lymph node status and ER, PR and Her-2/neu expression in present study. This was concurrent with finding of various studies.^{1,3,4,16,17} ER and PR expression was seen more in cases with grade I and II of Bloom Richardson grading whereas Her-2/neu positivity was seen more in cases with grade II and III of BR grading. Ambrose M et al (2011)³, Azizun Nisa et al (2008)¹ and Bhagat VM et al (2012)⁴ also reported the similar results. Expression of CK 5/6 was more associated with grade II and III of BR grading. Same finding were observed by Rattan et al. (2012)¹³ these finding was coincide with the finding of other studies.

This study reiterated that there should be a screening program about breast cancer and public health awareness programs through different modes of health education including self-palpation of breast to catch the disease in initial stage when it is curable. Molecular studies and immunohistochemical staining facilities are not available in public sector setup and high cost of such facilities in private setup is beyond the reach of poor patients of our country. So the Arrangements of such facilities at government institutions are recommended.

This study was concluded as ER and Her2/neu expression was found in 51.4%, PR expression in 47.1%, Ki-67 in 22.9% and

Cytokeratin 5 & 6 in 21.4%. In this study ER and PR expression was strongly associated. There were also an inverse correlation found in between Her-2/neu and ER, PR expression.

Tables:

Table 1: Showing association between various hormone receptors.

	ER	PR	Her 2/neu	Ki 67	Ck 5/6
ER	1#	0.861# (0.000)	-0.029# (0.806)	0.053# (0.660)	-0.328# (0.006)
PR		1#	-0.056# (0.642)	0.099# (0.406)	-0.284# (0.018)
Her 2/neu			1#	0.053# (0.660)	-0.328# (0.006)
Ki 67				1#	0.296# (0.013)
Cyt 5/6					1#

showed phi contingency coefficient value. Value in parenthesis showed p value.

Table 2: showing relation of different parameter with hormonal markers.

	ER +ve	PR +ve	Her 2/neu +ve	Ki 67 +ve	Ck 5/6 +ve
Age Group					
21 – 30 (6*)	3 (50)	2 (33.3)	2 (33.3)	0	1 (16.7)
31 – 40 (16*)	7 (43.8)	7 (43.9)	8 (50)	5 (31.3)	3 (18.8)
41 – 50 (26*)	13 (50)	12 (46.2)	16 (61.5)	7 (26.9)	5 (19.2)
51 – 60 (15*)	10 (66.7)	9 (60)	8 (53.3)	2 (13.3)	4 (26.7)
>60 (7*)	3 (42.9)	3 (42.9)	2 (28.6)	2 (28.6)	2 (28.6)
Site					
Left (48*)	27 (56.3)	25 (52.1)	24 (50)	11 (22.9)	11 (22.9)
Right (21*)	9 (40.9)	8 (36.4)	12 (54.5)	5 (22.7)	4 (18.2)
Tumor size group					
<2 cm.(T1) (1*)	1 (100)	1 (100)	0(0)	0 (0)	0 (0)
2-5 cm.(T2)(26*)	15 (57.7)	13 (50)	11 (42.3)	6 (23.1)	4 (15.4)
>5 cm. (T3)(43*)	20 (46.5)	19 (44.2)	25 (58.1)	10 (23.3)	11 (25.6)
Lymphnode involvement					
No LN (18*)	10 (55.6)	9 (50)	12 (66.7)	6 (33.3)	2 (11.1)
1-3 LN (23*)	14 (60.9)	13 (56.9)	8 (34.8)	4 (17.4)	3 (13)
>3 LN (29*)	12 (41.3)	11 (37.9)	16 (55.1)	6 (20.7)	10 (34.5)
Bloom Richardson grading					
Grade I (12*)	5 (41.7)	5 (41.7)	4 (33.3)	0	1 (8.3)
Grade II (42*)	27 (64.3)	25 (59.5)	23 (54.8)	9 (21.4)	9 (21.4)
Grade III (16*)	4 (25)	3 (18.8)	9 (56.3)	7 (43.8)	5 (31.3)

*were showed total number of cases in that group. Value in parenthesis showed percentage.

Table 3: Showing comparison of results of present study with others.

Author	ER (%)	PR (%)	Her2/neu (%)	Ki-67 (%)	CK 5& 6 (%)
Rhodes et al.(2000)11	77%	63%	---	---	----
Azizun- Nisa et al.(2008)1	32.7	25.3	24.7	-----	----
Munjal K et al.(2009)6	41.1	41.1	43	-----	----
Spitale A et al.(2009)12	85.3	71.9	85.3	---	----
Ambroise M et al .(2011)3	59	51	27	---	----
Baghat VM et al. (2012)4	48.27	37.93	27.58	---	----
Rattan B et al.(2012)13	17.9	----	20.5	17.9	----
Present study	51.4	47.1	51.4	22.9	21.4

Table 4: Showing Comparative finding of Present study with others

	Present Study	Ambroise M. et al. (2011)3	Rhodes A et al. (2000)11	Shet T et al. (2009)14
ER+ve / PR+ve	45.71%	47.0%	54.8%	41.8%
ER+ve / PR -ve	5.71%	12.2%	19.8%	10.6%
ER-ve / PR+ve	1.43%	4.05%	3.2%	3.4%
ER-ve / PR -ve	47.14%	36.8%	22.1%	44.2%

Fig 1: Showing IHC stain slide for Estrogen receptor quick score 8/8 - 40x

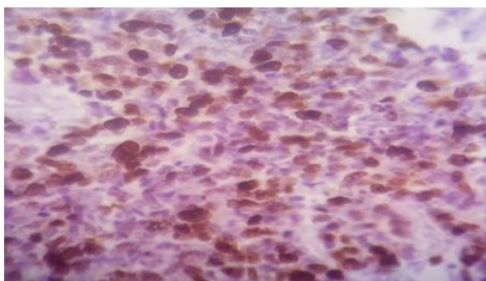


Fig 2: showing IHC stain slide for Progesterone receptor quick score 8/8 - 40x

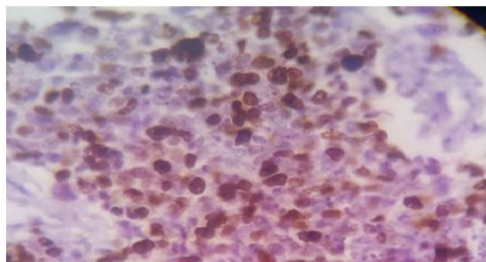


Fig 3: Showing IHC stain slide for HER2/neu score 3+ - 40x

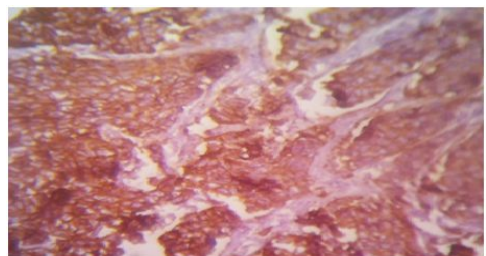


Fig 4: showing IHC stain slide for Ki-67 high score - 40x

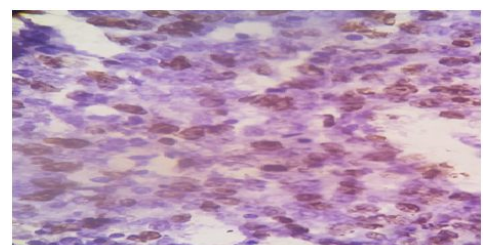
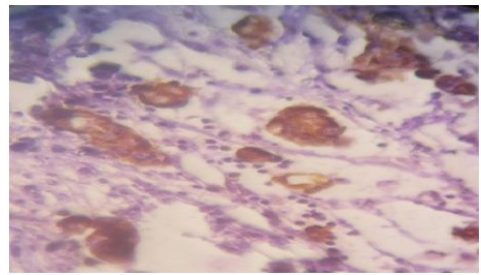


Fig 5: showing IHC stain slide for CK 5& 6 positive staining - 40x



Acknowledgement:

We are especially thankful to all the paramedical staff of the department, without their support this study cannot be completed.

REFERENCES

1. Azizun-Nisa, Yasmin Bhurgr, Farrukh Raza, Naila Kayani. Comparison of ER, PR & HER-2/neu (C-erb B Reactivity Pattern with Histologic Grade, Tumor Size and Lymph Node Status in Breast Cancer. Asian Pacific Journal of Cancer Prevention, 2008; 9:553-556.
2. Devi KU; Current status of gynaecological cancer care in India. J Gynecol Oncol., 2009; 20(2): 65-66.
3. Ambroise M, Ghosh M, Mallikarjuna VS, Kurian A; Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India. Asian Pacific Journal of Cancer Prevention, 2011; 12(3): 625-629.
4. Bhagat VM, Jha BM, Patel PR. Correlation Of Hormonal Receptor And Her-2/Neu Expression In Breast Cancer: A Study At Tertiary Care Hospital In South Gujarat. Nat. J. of Med. Research, 2012; 2(3): 295-8.
5. A. Sherif et al. Clinicopathologic Significance of Molecular Classification of Breast Cancer: Relation to Nottingham Prognosis Index. Journal of the Egyptian Nat. Cancer Inst., Dec 2010; 22(4): 209-215.
6. Munjal K, Ambaye A, Evans MF, Mitchell J, Nandedkar S, Cooper K; Immunohistochemical Analysis of ER, PR, Her2 and CK5/6 in Infiltrative Breast Carcinomas in Indian Patients. Asian Pacific Journal of Cancer Prevention, 2009; 10(5): 773-778.
7. Vaidyanathan K, Kumar P, Reddy CO, Deshmane V, Somasundaram K, Mukherjee G; ErbB-2 expression and its association with other biological parameter of breast cancer among Indian women. Indian Journal of Cancer, 2010; 47(1): 8-15.
8. Sandhu DS, Sandhu S, Karwasra RK, Marwah S. Profile of breast cancer patients at a tertiary care hospital in north India. Indian J Cancer, 2010; 47, 16-22.
9. Aryandono T, Harijadi, Soeripto. Hormone receptor status of operable breast cancers in Indonesia: correlation with other prognostic factors and survival. Asian Pac J Cancer Prev, 2006; 7, 321-4.
10. Taucher S, Rudas M, Mader RM, et al. Do we need HER- 2/neu testing for all patients with primary breast carcinoma? Cancer, 2003; 98, 2547-53.
11. Rhodes A, Jasani B, Balaton AJ, Barnes DM, Miller KD. Frequency of estrogen and progesterone receptor positivity in immunohistochemical analysis in 7016 breast carcinoma: correlation with patients age, assay sensitivity, threshold value, and mammographic screening. J Clin Pathol, 2000; 53, 688-96.
12. Spitale A, Mazzola P, Soldini D, Mazzucchelli L, Bordon A; Breast cancer classification according to immunohistochemical markers clinicopathologic features and short-term survival analysis in a population-based study from the South of Switzerland. Annals of Oncology, 2009; 20(4): 628-635.
13. Rattan B, Mridu M et al. The immunohistochemical expression of estrogen receptor, Her-2/neu and cytokeratin 8/18 and 5/6 in invasive breast carcinoma. Journal of clinical and diagnostic research, 2012; 6(9): 1495-1498.
14. Shet T, Agrawal A, Nadkarni M, Palkar M, Havaladar R, Parmar V; Hormone receptors over the last 8 years in a cancer referral center in India: what was and what is? Indian J Pathol Microbiol. 2009; 52(2): 171-174.
15. Almasri NM, Hamad M. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. Breast Cancer Research, 2005; 7(5): 598-604.
16. Mohammed RA, Ellis IO, Lee AH, Martin SG; Vascular invasion in breast cancer; an overview of recent prognostic developments and molecular pathophysiological mechanisms. Histopathology. 2009; 55:1-9.
17. Huang HJ, Neven P, Drijkoningen M, et al. Association between tumour characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. J Clin Pathol, 2005; 58, 611-6.