Original Research Article

Clinico-pathological co-relation using various immuno-histochemistry markers likeER, PR, HER-2 NEU, CK5/6, EGFR, KI-67 in carcinoma breast

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ABSTRACT

Introduction: In India, for the year 2012, 144,937 women were newly detected with breast cancer and 70,218 women died of it. For every 2 women newly diagnosed with breast cancer, one lady is dying of it. The aim of this study is to evaluate clinical parameters and pathological findings including various Immuno-histochemistry (IHC) markers like ER, PR, HER-2 NEU, CK5/6, EGFR, Ki-67 in cases of carcinoma breast and classify them into molecular classification based on IHC markers and try to correlate them clinically.

Materials and Methods: This prospective, observational study was carried out in 56 patients with early carcinoma breast (stage-I and stage-II) and IHC evaluation for various markers was done. Data was analysed by using Molecular Classification, divide them into estrogen positive (luminal HER-2, luminal A and luminal B) and estrogen negative (Triple negative or basal cell type, HER-2Neu type and normal breast like phenotype) subtypes. We had correlated this data with parameters like age of the patient, clinical and pathological staging of the breast carcinoma, presence or absence of nodes and presence or absence of other IHC parameters.

Results: We used ANOVA-F test to catagories variables and measure the test of significance. On IHC in Her-2 neu equivocal cases (patients who had two "++" positive points), we performed FISH test. Out of these 17 equivocal cases, only 3 were positive, 10 were negative and 4 patients did not underwent this test due to several reasons. Finally, Ki-67 value is significantly high in triple negative and Luminal-B patients. NPI is also having low 'P'value, although not reaching the level of significance.

Conclusion: Types of breast carcinoma, which look histologically similar behaves differently in their clinical presentation and in prognosis.In our study only Ki-67 was correlated with poor prognostic subtype of molecular classification but no any poor risk of clinical or histological parameter was correlated significantly with bad prognostic subtype of molecular classification as Luminal-B or triple negative type. We can say that this molecular classification is different in terms of prognosis in patients with similar looking clinical and histological parameters.

1. Introduction

Worldwide, breast cancer is by far the most common cancer amongst women, with an incidence rate more than twice that of colorectal cancer and cervical cancer and about three times that of lung cancer. However breast cancer mortality worldwide is just 25% greater than that of lung cancer in women (WHO, 2003).[1] In 2004, breast cancer caused 519,000 deaths worldwide (7% of cancer deaths; almost 1% of all deaths).[2] In India, for the year 2012, 144,937 women were newly detected with breast cancer and 70,218 women died of it. In India, for every 2 women newly diagnosed with breast cancer, one lady is dying of it. In comparison, in USA in the year 2012, incidence was 232,714 with 43,909 death and one death for 5-6 breast carcinoma patients and in China in year 2012, incidence was 187,213, with 47,984 death and one death for 4 breast carcinoma patients. Since

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more patients (in India) turn up in later stages, they do not survive long irrespective of the best treatment they may get, and hence the mortality is fairly high. There are lots of reasons for late presentations including lack of awareness, shyness on part of patients, social stigma, ignorance of doctors (patients present on time, but doctors are not aware and they delay treatment), and many other causes.

The aim of this prospective study is to evaluate clinical parameters and pathological findings including various Immuno-histochemistry (IHC) markers like ER, PR, HER-2 NEU, CK5/6, EGFR, Ki-67 in cases of carcinoma breast in India. These aims shall be fulfilled with the help of following objectives:

1. To study the clinical and pathological profile of patients of carcinoma breast enrolled in the study.
2. To carry out Immunohistochemical investigations like ER, PR, HER-2 NEU, CK 5/6, EGFR and Ki-67 on cancer tissue.

And, classify them into molecular classification based on IHC markers and try to correlate them clinically. Most of the studies with IHC markers have been carried out in western population. In Indian subcontinant data from IHC based studies in carcinoma breast is sparse. Considering these facts, the proposed study shall try to evaluate the role of IHC markers in identification, classification and established clinic-pathological correlation in cases of carcinoma breast in North Indian popolation.

2. Material and Methods

The present study has been carried out in the Department of Surgical Oncology in collaboration with Department of Pathology in Dharamshila Hospital & Research Centre, Delhi. This prospective and observational study was carried out in patients of carcinoma breast attending Surgical Oncology OPD. Total 56 only female patients with early carcinoma breast (stage-I and stage-II) undergoing upfront surgery with or without reconstruction were included in the study.

2.1. Immunohistochemical Evaluation

For ER/PR positivity, HER-2 NEU, CK5/6, EGFR, Ki-67 positivity and expression level was done using standardized laboratory techniques by the dept. of pathology in DHRC.

We did IHC by manual method and reagents as PAP and antibodies by Thomas Boenisch, editor director immunohis-tochemistry laboratory DAKO corporation, Santa Borbora, California (Bio genex laboratories). This method permits the specific demonstration of cells and tissue antigens in a variety of fixed tissues.

2.2. Data analysis

After getting all the information, we analysed the data and by using Molecular Classification given by Perae et al[3] divide them into estrogen positive (luminal HER-2, luminal A and luminal B) and estrogen negative (Triple negative or basal cell type, HER-2Neu type and normal breast like phenotype) subtypes

2.3. Luminal A tumors

These are ER positive, PR positive or negative, HER2 negative, and CK5/6 and EGFR negative.[4,5]

2.4. Luminal B tumors

They are ER positive and either HER-2 Neu positive or having high Ki-67 index ($\geq 15\%$).[6]

2.5. HER-2 Neu type

ER, PR negative and HER-2 Neu positive.[7]

2.6. Triple negative

ER, PR and HER-2 Neu negative and CK5/6 or EGFR positive.[3]

2.7. Normal Breast Like(NBL)

ALL 5 markers are negative.[8]

We have correlated this data with parameters like age of the patient (whether poor risk factors are present in younger patients or not), clinical and pathological staging of the breast carcinoma, presence or absence of nodes and presence or absence of other immunohistochemical parameters.

3. Results

As we have divided our group into 5 catagories, we used ANOVA-F test to catagorise variables and measure the test of significance. In these results Ki-67 value is significantly high in triple negative and Luminal-B patients. NPI is also having low 'P'value, although not reaching the level of significance.

Initially we included 60 patients. On IHC in Her-2 neu equivocal cases (patients who had two "++" positive points), we performed FISH test. Out of these 17 equivocal cases, only 3 were positive, 10 were negative and 4 patients did not underwent this test due to several reasons, as one patient did not want to take Herceptin due to her age, two had financial issues and other had changed the hospital. So finally 56 patinets were included in the study.

Table 1:

Variable	All cases N=56(%)	Luminal A N=24(%)	Luminal B/Luminal–HER2 hybrids N=8(%)	Her2/neu type N=9(%)	Triple negative N=8(%)	Normal Breast Like (NBL) N=7	P value
1.Age-specific groups, Mean Age	**53.54**	**55.91**	**46.25**	**51.67**	**54.87**	**51.71**	0.536
<50	21(37.5)	7(29.16)	6 (75%)	3(33.3)	1(12.5)	4(57.1)	
50-69	29(51.8)	13(54.16)	2(25)	6(66.67)	6(75)	2(28.6)	
≥70	06(10.7)	4(16.66)	0	0	1(12.5)	1(14.3)	
2. Premenopausal	19(33.9)	8(33.33)	5(62.5)	2(22.2)	1(12.5)	3(42..8)	0.443
3.Postmenopausal	37(66.1)	16(66.67)	3(37.5)	7(77.8)	7(87.5)	4(57.2)	
4.Laterality, n (%)							
Right	26(46.4)	11(45.83)	5(62.5)	3(33.3)	4(50)	3(42..8)	0.405
Left	30(53.6)	13(54.17)	3(37.5)	6(66.67)	4(50)	4(57.2)	
5.Dietary Factors							
Non Veg	23(41.1)	9(37.5)	2(25)	5(55.6)	5(62.5)	2(28.6)	0.323
Veg	33(58.9)	15(62.5)	6(75)	4(44.4)	3(37.5)	5(71.4)	
6.BMI(Kg/m2) Mean	**26.42**	**27.48**	**27.04**	**25.34**	**24.40**	**26.82**	0.552
<25,n(%)	22(39.2)	9(37.5)	3(37.5)	4(44.4)	5(62.5)	1(14.3)	
≥25,n(%)	34(60.8)	15(62.5)	5(62.5)	5(55.6)	3(37.5)	6(85.7)	
7.Tumor size(cm)	3.07	2.7	3.63	3.5	3.15	3.37	0.322
≤ 2 cm	12(21.4)	10(41.67)	0	1(11.1)	1(12.5)	0	
>2-5 cm	39(69.7)	12(50)	7(87.5)	7 (77.8)	6 (75)	7(100)	
>5 cm	5(8.9)	2(8.33)	1(12.5)	1(11.1)	1(12.5)	0	
8. Histological Type							
IDC	42(75.0)	15(62.5)	8(100)	7(77.8)	7(87.5)	5(71.4)	
I. Lobular Ca.	8(14.3)	5(20.8)	0	2(22.2)	0	1(14.3)	
Others	6(10.7)	4(16.66)	0	0	1(12.5)	1(14.3)	
9. Histologic grade (Elston/Ellis), n (%)	**2.55**	**2.29**	**2.75**	**2.55**	**3.0**	**2.42**	0.20
Grade I	3(5.3)	3(12.5)	0	0	0	0	
Grade II	21(37.5)	11(45.83)	2(25)	4(44.4)	0	4(57.2)	
Grade III	32(57.2)	10(41.67)	6(75)	5(55.6)	8(100)	3(42..8)	
10.Lymph node status, n (%)							0.241
Positive	23(41.1)	9(37.5)	4(50)	5(55.6)	3(37.5)	2(28.6)	
Negative	33(58.9)	15(62.5)	4(50)	4(44.4)	5(62.5)	5(71.4)	
11. EIC	8 (14.3)	3(12.5)	2(25)	2(22.2)	1(12.5)	0	
12. LVI	21(37.5)	7(29.16)	3(37.5)	5(55.6)	5(62.5)	1(14.3)	
13. NPI	4.85	4.32	5.47	5.02	5.13	4.66	**0.149**
14. ER/PR status							
Positive	32(57.1)	24(100)	8(100)	0	0	0	
Negative	24(42.9)	0	0	9(100)	8(100)	7(100)	
15. HER 2-Neu							
Positive	14(25.0)	0	5(62.5)	9(100)	0	0	
Negative	42(75.0)	24(100)	3(37.5)	0	8(100)	7(100)	
16. Ki-67 index, n (%)	**11.16%**	**6.45%**	**18.12%**	**6.67%**	**25.62%**	**10.7%**	**0.001**
<10%	46(82.1)	24	4(50)	8(88.9)	4(50)	6(85.7)	
≥15%	10(17.9)	0	4(50)	1(11.1)	4(50)	1(14.3)	

4. Discussion

Breast carcinoma is a heterogenous disease and it behaves differently in different groups of populations. Previously it was seen that breast cancer was common in developed countries and cervical cancer was most common in developing countries like India. Now, we have seen that incidence of breast cancer is increasing in our country for the last decade and it is becoming the most common cancer in females. Breast cancer shows clinical and morphological diversities and variability in prognosis and response to different therapeutic modalities. The existing histological classification systems for breast cancer are far from being accurate in predicting the prognosis or selecting the appropriate treatment of a given patient.[9] That is why there may be a need for a different classification system as molecular classification. This would result in less frequent use of chemotherapy with considerable advantages in reducing toxicity and costs.[10] Perou et al were the first to provide a classification system based on gene expression analysis, and this consisted of four major molecular classes of breast cancer: luminal-like, HER-2 positive, basal-like and normal-like.[3] Subsequent studies suggested the existence of more molecular classes and this ultimately led to addition of a fifth category, with the molecular spectrum now expanding to luminal A (LUM-A), luminal B (LUM-B), HER2 over expressing, basal-like, and normal-like.[11] A further advancement in the field was the use of IHC as a surrogate for DNA microarray classification. Studies confirmed that it could reliably identify the major molecular classes of invasive breast carcinoma. This method represents a feasible alternative because many of the cases of breast cancer occur in places where analysis of prognostic factors needs to be economical, easy and reproducible.[6]

Recently published studies have used five surrogate IHC markers (ER, PR, HER2, CK5/6, and EGFR) for molecular class distinction. We have used six markers including Ki-67 in addition, to differentiate the luminal-A and luminal-B. Luminal tumors being categorized by hormone receptor (HR) positivity and/or HER2 expression, a feature of HER 2 tumor.[12,13] At least five main molecular classes of breast cancer are currently recognized: as Luminal A, Luminal B, HER2, Basal, Unclassified.

Luminal A tumors are ER positive, PR positive or negative, HER2 negative, and CK5/6 and EGFR negative.[4,5] Luminal A is the most frequent subtype. It shows a good prognosis and responds well to hormone therapy. Various studies have reported that ER+ tumors have little response to conventional chemotherapy. It has been demonstrated that patients with ER- tumors have more complete pathological responses to neoadjuvant chemotherapy than ER+ tumors.[14]

Luminal B tumors are ER positive and either HER-2 Neu positive or having high Ki-67 index ($\geq$ 15%)[6]. Identification of luminal B tumors at the protein level is a point of controversy. Some authors have used the co-expression of HR and HER2 to define this group, based on the fact that the HER2-associated genes (i.e., ERBB2 and GRB7) are expressed in 30-50% of luminal tumors.[6] However, these tumors have a poorer prognosis than Luminal-A tumors and are endocrine (tamoxifen) resistant and require blockage of HER2 pathways in addition to estrogen deprivation.[15] Therefore, including them as an integral component of endocrine-sensitive luminal tumors may not be justified. Due to these complexities, the HER2+ tumors need to be considered separately from pure luminal tumors, which should be further categorized as luminal A and luminal B, with those showing co-positivity of HER2 grouped into a separate hybrid category termed "luminal–HER2 hybrids."[4,5] We have taken Luminal-B and HER-2 hybrid group together.

Bhargava et al[5] defined Luminal-A and Luminal-B as pure hormone receptor positive, the differentiating feature between them being the strong intensity of ER positivity in Luminal-A tumors. So there is still some controversies in defining these groups.

HER2+ tumors are HER2 positive, ER and PR negative, and CK5/6 and EGFR negative.[5] The poor prognosis of HER2 originates in its high risk of early relapse.[7] Basal-like tumors are CK5/6 and/or EGFR positive, ER and PR negative, and HER2 negative. The basal class is so named due to its pattern of expression that is similar to basal epithelial cells and normal myoepithelial cells of mammary tissue.[16] This similarity is a product of the lack of ER expression and related genes, low expression of HER2, intense expression of CKs 5, 6, and 17, and the gene expression related with cellular proliferation.[6] Using IHC, this class has also been called "triple negative" for not expressing ER, PR, or HER2. It has been associated with the BRCA1 mutation.[17]

Unclassified (penta −ve) tumors are ER and PR negative, HER2 negative, and CK5/6 and EGFR negative. They correspond to those triple-negative tumors not exhibiting basal markers. Unclassified cases were initially considered to be synonymous with "normal-like" breast cancers. These tumors cluster with non-tumoral breast cells and exhibit overexpression of PIK3R1 and AKR1C1, in addition to other genomic alterations.[18] The current concept states that the "normal-like" subtype is absolutely different from the unclassified (penta −ve) "ER−, PR−, HER2−, and CK5/6 and EGFR−" group, as absent or decreased expression of basal markers is not a feature compatible with the "normal-like" molecular class.[8] They are very good prognostically and are grouped with the luminals.[19] The unclassified and "normal-like" are completely separate entities and IHC surrogates for these categories have not yet been developed. Associating these with a particular set of negative or absent markers may lead to misinterpretations of their intrinsic biological characteristics.[20]

Types of breast carcinoma, which look histologically similar behaves differently in their clinical presentation and in prognosis. As we all know that some breast cancer patients with low histologocal score may present with upfront meatastatic disease or they are resistant to standard chemotherapy or presents with early recurrence after completion of treatment. Even some patients with aggressive tumors (poorly differentiated and high histological scores) may have complete treatment response and enjoy the long term survival without recurrence or metastasis. That is why, it was thought that there was something we were missing in the histological classification (on light microscopy) of breast carcinoma, which we did not recognize and that affects the overall prognosis. After this molecular classification had come. In our study only Ki-67 was correlated with poor prognostic subtype of molecular classification but no any poor risk of clinical or histological parameter was correlated significantly with bad prognostic subtype of molecular classification as Luminal-B or triple negative type. We can say that this molecular classification is different in terms of prognosis in patients with similar looking clinical and histological parameters. Oncotype DX and Mamma Print are now in the clinical use although these tests are costly and still not done frequently in developing countries, in future these tests will be available more frequently.

Presently, molecular classification of breast carcinoma does provide additional prognostic and predictive information to clinical and pathological features, alone by which, it is difficult to predict the prognosis. Many targeted drugs can be used with fewer side effects; also chemotherapy can be modified accordingly. However, this benefits a limited numbers of the patients and mainly restricted to the ER positive (Luminal –A) patients. Also, there is some controversy in this classification, as in Luminal B and how to differentiate basal types from pure breast like. But in future this will be resolved and there will be less need to take chemotherapy and patients will enjoy the treatment mainly based on targeted therapy.

5. Source of funding

None.

6. Conflict of interest

None.

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