Original Article

ER, PR HER2-neu Study in Breast Cancer Patients of Southern Rajasthan

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Background : Breast Carcinoma is the most common malignancy in Indian females, It is also the most common Female Cancer World wide with estimated incidence of around 25% among all Cancers. For targeted therapy breast carcinomas are further classified on the basis of their molecular profile. This molecular classification is becoming the gold standard for complete characterization of Breast Cancer. But in resourse limited settings Tumor markers ie, ER, PR and HER2, are routinely available in Breast Cancer specimens, are reliable, inexpensive and useful for therapeutic decision making.

Aims & Objective : To evaluate ER, PR HER2-neu status of patients with Breast Carcinoma. To improve prognostic value and aim at targeted therapy.

Material and Method : The study was conducted on 100 proven Breast Cancer patients of Udaipur and surrounding tribal belt attending Mahrana Bhupal Government Hospital, Udaipur. IHC was done on fully automated IHC instrument leica bond max and slides were prepared. Slides were examined and scoring was done by allred method for ER/PR and for Her2 scoring was done according to ASCO/CAP scoring staining pattern.

Results : Patients were divided in four major groups on the basis of IHC ie, ER/PR$^+$ & Her2$^+$, ER/PR$^+$ & Her2 -ve, Triple negative and Her2 Overexpressed. Hormone positivity was seen among 54.63% cases and most of them were grade II histolgically. Most of the patients were in 41 to 60 years of age group.

Conclusion : IHC markers are helpful in guiding for treatment protocols in Breast Cancer patients and help in stratifying the patients in different risk group according to their prognosis.

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Key words **: IHC, Breast cancer.**

Breast carcinoma is the most common malignancy in Indian females with age adjusted rate as high as 25.8 per 100,000 women and mortality rate around 12.7 per 100,000 women. It is also the most common Female Cancer world wide with estimated incidence of around 25% and 2.3 million newly diagnosed cases in year 2020[1]. The increasing incidence and decreased 5 year survival rate has been attributed to change in lifestyle, late marriage, decreased breast feeding, lack of screening, late presentation, delayed and incomplete treatment.

Surgery has been the mainstay of treatment but now due to increasing awareness and availability about targeted therapy, efforts must be put to prolong the survival and improve the outcome. For targeted therapy Breast Carcinomas are further classified on the basis of their molecular profile.This molecular classification is becoming the gold standard for complete characterization of Breast Cancer.

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Editor's Comment :

- Breast carcinoma is one of the most common malignancy and ER PR and Her2 testing helps in determining the prognosis and giving targeted therapy to patients.

However, for molecular classification gene expression profiling, a high end technology is required so Clinicians usually rely on clinicomorphological pattern and readily available tumor markers which act as surrogate marker for molecular profiling.

These Tumour markers ie, ER, PR and HER2, are routinely available in Breast Cancer specimens, are reliable, inexpensive and useful for therapeutic decision making.

Immuno-histochemistry for these Tumour markers has been very important for deciding prognosis, predicting response to therapy and evaluating residual Tumour cells in post treatment cases.

Aims and Objectives

To evaluate ER, PR HER2-neu status of patients with Breast Carcinoma .

To improve prognostic value and aim at targeted therapy.

Materials and Methods

The study was conducted on 100 proven Breast Cancer patients of Udaipur and surrounding tribal belt attending Mahrana Bhupal Government Hospital, Udaipur.

Both the specimen and prepared blocks were accepted for study of Tumour markers in Breast carcinoma patients. Detail clinical history and relevant clinical information was recorded in pre-designed performa.

IHC was done on fully automated IHC instrument leica bond max and slides were prepared (Table 1).

Slides were examined and scoring was done by allred method for ER/PR and for Her2 scoring was done according to ASCO/CAP scoring staining pattern.

Patient were followed up quarterly for one year regarding treatment and further workup, records were maintained (Fig 1).

Results

We included a total of 100 patients in our study, out of which only one patient was Male rest all were Female this was an expected finding as the incidence of Breast Carcinoma in males worldwide is only 0.5 to 1%[4]. Out of total 100 cases results were acceptable in 97cases as in three cases due to tissue loss during processing results were rejected.

Most common age group involved is 40-60 years with 80.41% patients falling in this age group. Most of the Tumours were Invasive Ductal Carcinoma with No special type (95.8%) and Grade II was predominant histological grade.

We divided our patients in four major groups on the basis of IHC ie, ER/PR$^+$ & Her2$^+$, ER/PR$^+$ & Her2 -ve, Triple negative and Her2 Overexpressed. Hormone positivity was seen among 54.63% cases and most of them were grade II histolgically.

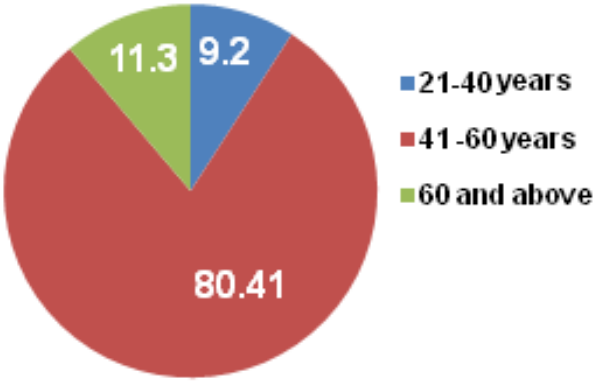

Fig 1 — Pie diagram for %incidence in various age groups

One year follow-up was done for completion of treatment, recurrence and mortality which showed

That 8 patients died during 12 months, out of which 3 had stopped treatment in between while rest 5 were either on treatment or completed the Chemotherapy cycles. 4 out of these 5 patients were triple negative on IHC. Mortality in Triple Negative (TN) group was highest and all 8 patients were in age group of 61 and above.

Discussion

The incidence of Breast Cancer is increasing Globally, with an extra surge in Asian countries, especially in pre-menopausal women. Breast Cancers are multifaceted disease with different morphologies and biological behaviors. Gene expression profiling studies have identified at least four categories of Breast Cancer: Luminal A, Luminal B, HER2 overexpressing, and basal-like or Triple Negative (TN)[2]. These molecular categories have been correlated with Immuno-histochemical (IHC) biomarkers[3].

In our study most of the patients were in the age of 41-60 years which is also noted in many previous studies as most Indian studies have recorded median ages ranging from 48-53 years[4-6]. In comparison, the median age at diagnosis for Cancer of the Breast in the US is 61 years[7].

It can be assumed that the actual age of onset of Breast Carcinoma in the Indian patient is lower by well over a decade& this younger age of onset of Breast Cancer can be explained by racial differences.

Table1 — *Morphological spectrum of breast cancer patients and their IHC profile*

	ER/PR$^+$ Her2 -ve	ER/PR$^+$ Her2 +ve	ER/PR -ve Her2 -ve	Her2 Overexpressed
Median age	42.9±14.6	55.9±12.5	58.1±14.7	59.9±12.8
Histological grade :				
Grade I	02(2.06%)	01(1.08%)	NIL(0.00%)	NIL(0.00%)
Grade II	26(26.80)	18(18.55%)	16(16.49%)	16(16.49%)
Grade III	06(6.1%)	NIL(0.00%)	17(17.52%)	05(5.15%)
Tumour size :				
<2 cm	22(64.7%)	05(26.3%)	01(3.03%)	02(9.5%)
2-5cm	07(20.5%)	14(73.6%)	15(45.4%)	09(42.8%)
>5.0cm	05(14.7%)	NIL	14(42.4%)	08(38.09%)
NA	NIL	NIL	03(9.09%)	02(9.52%)
Lymphnode status :				
Positive	10(29.41%)	06(31.5%)	02(6.06%)	04(19.04%)
Negative	24(70.58%)	13(68.42%)	28(84.8%)	15(71.42%)
NA	NIL	NIL	03(9.09%)	02(9.52%)

Now-a-days targeted therapy is the mainstay for treatment of Cancers & IHC and molecular studies are required for diagnosis, prediction, treatment and prognostication of cancers at any site[8].

Breast cancer has been divided into six molecular subtypes : Luminal A, Luminal B, basal like, HER2 like, normal epithelial like and claudin low[9]. However, The IHC surrogates for the molecular subtypes are: Luminal A (ER^+ or PR^+ or both, HER2 neu negative), Luminal B (ER^+ or PR^+ or both, HER2 neu^+) or (ER^+, low PR^+, HER2-neu, high Ki67), basal like (ER^-, PR^-, HER2 neu±), HER2-neu^+ (ER^-, PR^-, HER2-neu^+). Any degree of Hormone receptor positivity makes the patient ideal candidate for Hormone therapy.

In our study 54.63% cases were positive for Hormone receptor out of which most were grade II (81.13%) this was in concordance with Kumar, *et al* however, the median age was lower than their study. Amongst these patients all were positive for ER however 17.8% were negative for PR.

ER expression has been labeled as a good prognostic and predictive biological marker through various studies and is associated better overall survival compared to ER negative Tumours.

However, independent prognostive and predictive role of PR expression irrespective of ER has been matter of great debate. ATAC (Arimidex, Tamoxifen, Alone or in Combination) adjuvant trial compared the efficacy of tamoxifen with that of the aromatase inhibitor anastrazole_showed that patients with ER^+/PR^+ Tumours had a lower recurrence rate than those with ER^+/PR^- tumors (7.6% *versus* 14.8%, respectively)[11].

Triple Negative Breast Carcinomas mainly of high histologic grade (grade III), showed high mitotic index and are found more frequently in pre-menopausal women. In our study median age of this group was 56±2 years with most of them falling in histological grade III.

Study conducted by Umemura and colleagues found that combined estrogen receptor-negative and HER2-negative Tumours constitute 19% of cases (11 of 58 Breast Cancer cases) & were associated with high expression of p53, vimentin and EGFR and these tumours showed the highest ki-67 Labeling Index and lowest expression of cyclinD1 when compared with other tumour groups[12].

The overall survival was least in this subgroup with 12.12% mortality in 12 month follow-up period so triple negative group has the worst overall and disease-free survival while overall survival was good in Hormone positive & HER2 negative subgroup which is in concordance with preivous studies[13-15].

Her2 Overexpression has both prognostic and predictive implications and the incidence of Her2 Overexpression is around 15-30% in Invasive Breast Cancers[16]. Trastuzumab was approved as part of a treatment regimen containing doxorubicin, cyclophosphamide, and paclitaxel for the adjuvant treatment of women with node-positive, HER2 overexpressing Breast Cancer.

In our study Her 2 score 3 as per CAP guidelines was reported as positive, score 2 was given equivocal and as National Comprehensive Cancer Network (NCCN) guidelines panel recommended that less than 3^+ overexpression of HER2-neu by IHC should be additionally examined by FISH or other in situ hybridization methods so these cases were advised for further FISH testing, while score 0 and 1 was reported as Negative.

We had a total of 21.61% cases in this subgroup. When compared to Hormone positive subgroup patients in this group has higher histological grade and higher stage.

Though Recent publications have shown that newer molecular classification of Breast Cancer have greater prognostic value & Subtyping Breast Cancer using microarrays for gene expression analysis is the ideal method for such molecular classification but the availability and cost of these test are genuine constrains.

For such instances IHC-based classification systems are very useful and has been shown to correlate well with intrinsic classification using gene expression microarrays.

IHC system has it's own limitation as there is intralaboratory and interlaboratory variation in ER results because fixation, antigen retrieval, and staining methods may differ among laboratories[17].

Similary discordance among Her2 results generated in different laboratories from the same specimen has also been reported[18].

Limitations of the study :

Study was conducted on a small group of patients and follow up period was also short . Her2 euqivocal caeses were followed for their FISH results but due to cost constrains most were either lost during follow up or such cases ended up for routine Chemotherapy due to absence of definiative HeR2 status.

Conclusion :

The biology and complex genomic intricacies of Breast Carcinoma has categorized it into different molecular subtypes but for countries with limited resources IHC can still be considered a valuable tool

for Clinicians which is simple, inexpensive, easy to interpret,reliable, reproducible and readily available.

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