



EVALUATION OF HER-2/neu STATUS IN CARCINOMA BREAST SPECIMENS USING IMMUNO-HISTOCHEMISTRY (IHC) & FLUORESCENCE IN -SITU HYBRIDISATION (FISH) ASSAY

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ABSTRACT Breast cancer is the second most common cancer in the world and by far the most frequent cancer among women. Among the various prognostic and predictive factors of breast cancer, the most widely studied biomarker is the human epidermal growth factor receptor 2 (HER-2) gene, also referred to as *ERBB2/erbb2* or HER-2/neu by IHC & FISH.

AIMS AND OBJECTIVES: To evaluate the association between HER-2/neu status and Comparison of Fluorescence In Situ Hybridization an Immunohistochemistry.

MATERIALS & METHODS The present study was conducted at the Department of Pathology, Kurnool Medical College, Kurnool. It is a prospective study done between June 2014 to June 2016. The study material consists of 60 cases of mastectomy specimens of breast carcinoma which were selected for HER-2/neu status by IHC & FISH.

RESULTS: Immunohistochemistry for HER2/neu as per American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines revealed that 18(30%) cases were HER-2/neu positive, 9(15%) were equivocal and 33 cases (55%) were negative.

CONCLUSION In conclusion, in the present study both IHC and FISH were employed for determination of HER-2/neu status in invasive breast carcinoma. With most authorities agreeing upon FISH to be more reliable than IHC for determining the HER-2 status

KEYWORDS : Breast, carcinoma, HER2/neu, immunohistochemistry, FISH.

INTRODUCTION

Breast cancer is the second most common cancer in the world and by far the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). So roughly, in India, one out of 2 women newly diagnosed with breast cancer, is dying of it. It is the second most common cancer among women in India and accounts for 7% of global burden of breast cancer and one-fifth of all cancers among women in India cancer among women in India and accounts for 7% of global burden of breast cancer and one-fifth of all cancers among women in India¹ By 2020 breast cancer is set to overtake cervical cancer as the most common type of cancer among all women in India.

Among the various prognostic and predictive factors of breast cancer, the most widely studied biomarker is the human epidermal growth factor receptor 2 (HER-2) gene, also referred to as *ERBB2/erbb2* or HER-2/neu. HER2/neu play an important role as a prognostic and therapeutic tool in breast cancer. The human epidermal growth factor receptor 2 (HER-2) gene, otherwise known as C-erb-B2 is amplified in approximately 18–20% of breast cancer². Fluorescence *in situ* hybridization (FISH) is being recognized as the most accurate and predictive test for HER2/neu gene amplification and response to therapy in breast cancer. HER-2/neu, gene amplification by FISH in breast carcinoma specimens and compared the results of FISH analysis with IHC analysis. HER-2/neu amplification has been associated with a number of adverse outcomes, including decreased overall and disease-free survival³.

AIMS AND OBJECTIVES

- To study the incidence and age wise occurrence of breast Carcinoma in tertiary care hospital
- To evaluate the HER-2/neu status in breast cancer specimens using IHC and FISH assays.

MATERIALS & METHODS

The present study was conducted at the Department of Pathology, Kurnool Medical College, Kurnool. It is a prospective study done between June 2014 to June 2017.

- The study material comprised of 60 cases of mastectomy specimens operated for breast carcinoma. Best sections representing the tumor were selected for HER-2/neu status by immunohistochemistry and FISH analysis. After blocking the endogenous peroxidase activity with 0.3% hydrogen peroxide for 5 to

10 minutes, the sections were stained for HER-2/neu using polyclonal anti-rabbit HER-2 antibody according to instructions of the manufacturer and using reagents included in HER-2/neu detection kit (DAKO). Scoring of HER-2/neu was done as per American Society of Clinical Oncology College of American Pathologists (ASCO/CAP) guidelines for IHC scoring. Positive HER-2/neu staining was identified in the form of membrane staining.

Each slide was scored in a blinded fashion by two pathologists according to the manufacturer's recommended criteria. Intensity scores of 0 or 1+ were designated as negative expression and 3+ were designated as positive expression for HER-2/neu. Scores of 2+ were taken as equivocal Cases/ inconclusive. Fluorescence in-situ hybridization (FISH) was performed using FDA approved Path-Vysion HER-2/neu DNA probe kit (Abott, Molecular Inc., IL, USA) which contained two labeled DNA probes: the HER-2/neu probe which comprises locus specific identifier (LSI) HER-2/neu gene labeled in spectrum orange, and chromosome enumeration probe 17 (CEP 17) which is labeled in spectrum green and hybridizes to the alpha satellite DNA located at the centromere of chromosome 17(17p11.1). The polysomy 17 was also recorded in the cells as four spec green signals as moderate polysomy and >4 spec green signals as high polysomy.

RESULTS;

The present study is a two year prospective study of 60 cases of carcinoma breast, which presented to the Department of pathology, Government General Hospital, Kurnool from June 2014 to June 2016. All 60 cases were evaluated with subsequent IHC marker study and FISH for HER-2/neu. The following observations were made.

Table 1: Incidence of Breast Lesion

YEAR	TOTAL BIOPSIES	BREAST LESIONS	BENIGN	MALIGNANT
June 2014- May 2015	2812	61	34	27
June 2015- June 2016	3200	86	53	33

In the present study age of the individuals with carcinoma breast ranged from 26 to 80 years with mean age of group of 49.73 yrs. Maximum number of cases were seen between 51-60 yrs(40%), followed by the age group in the range of 41-50 yrs(28.3%), and 21.6% between 31-40 yrs

Immunohistochemistry for HER2/neu as per American Society of Clinical Oncology / College of American Pathologists (ASCO /CAP) guidelines revealed that 18(30%) cases were HER-2/neu positive, 9(15%) were equivocal and 33 cases (55%) were negative.

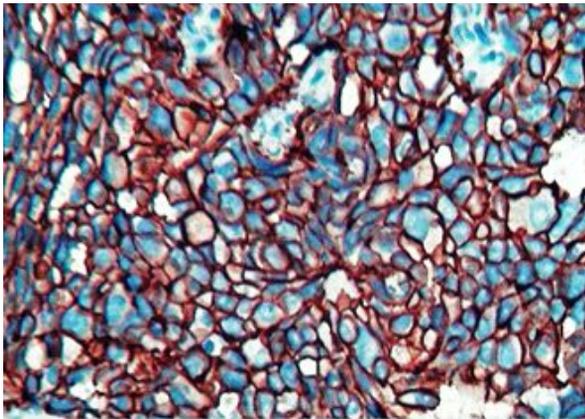
Incidence of HER-2/neu positive tumors was more in <50yrs age group, 11(35.5%).

HER-2/neu had got no statistical correlation with Age. (P value= 0.3378).

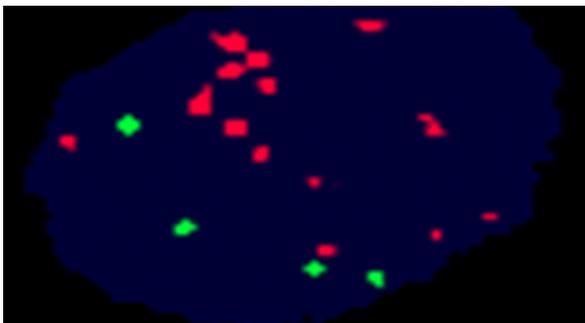
Table:3 Comparison of IHC results with FISH

HER2 protein expression by IHC	HER2 testing by FISH	
	AMPLIFIED (+ve)	NON AMPLIFIED (-ve)
NEGATIVE(0/1+)N=33	5(15.2%)	28(84.8%)
EQUIVOCAL(2+)N=9	5(55.5%)	4(44.5%)
POSITIVE(3+)N=18	17(94.4%)	1(5.6%)
N= 60	27	33

In the present study 17 out of 18 IHC Positive (3+) cases showed gene amplification accounting to 94.4%, 5 out of 9 IHC equivocal (2+) cases showed gene amplification accounting to 55.5%, 5 out of 33 IHC negative (1+/0) cases showed gene amplification accounting to 15.2%



HER-2/neu score 3+ by IHC



Cell showing four green and multiple orange signals. POSITIVE FOR HER-2/neu GENE AMPLIFICATION by FISH

DISCUSSION

The present study is a prospective study performed at the Dept of Pathology, Kurnool Medical College, Kurnool from June, 2014 to June 2016.. It consists of 60 cases of breast carcinoma evaluated histopathologically by H & E stain, followed by IHC & FISH studies for HER-2/neu. Cases with IHC reactions of 2+ for HER-2/neu were considered inconclusive/equivocal. Thus the present study reveals evaluation of HER-2/neu status by IHC and comparing it with FISH results. Age of the cancer patient is an important factor both for occurrence and management of the case. In the present study the mean age of the patient was 49.7 yrs. As per the SEER cancer statistics review 1975- 2004 of USA, the mean age of a breast cancer patient was 61.0 yrs. The average age of occurrence of the breast cancer in India reveals that the disease occurs a decade earlier, as compared to western countries¹

HER-2/neu scoring done as per ASCO/CAP guidelines. In the present study 18 out of 60 cases (30%) showed HER-2/neu positivity (3+) which is similar to kalar Iravathy Goud et al, Panjawani et al study which showed 35.5% and 46.8% respectively. In the present study 33 out of 60 cases (55%) showed HER-2/neu negativity(0/1+) which showed discrepancy with Ridolfi et al study in which 588 out of 750 cases showed HER-2/neu negativity accounting for 78.4% of total study population. It is well known that HER-2 positivity rates in breast cancer varies from western population to Asian population. In the present study 27 out of 60 cases showed HER-2/neu gene amplification accounting to 45% which is similar to Kalar Iravathy Goud et al study. None of the cases showed equivocal results. Only one case showed polysomy of chromosome 17 with co-existent gene amplification accounting for 1.6%

Table 4: Comparison of HER-2/neu status by IHC with FISH with other studies.

	Present study		Panjawani et al study ⁶⁶		Kalar iravathy goud et al ⁶⁴	
	IHC	FISH (Amplified)	IHC	FISH (Amplified)	IHC	FISH (Amplified)
Positive (3+)	18	17 (94.4%)	82	77 (93.9%)	32	25 (78.1%)
Equivocal (2+)	9	5 (55.5%)	36	24 (66.6%)	28	25 (89.2%)
Negative (1+/0)	33	5 (15.2%)	57	7 (12.2%)	30	-

Although there is good correlation between HER-2 gene amplification and protein overexpression, many studies also have shown that 3 to 15 per cent of breast carcinomas over- express the HER-2 protein without gene amplification and a small subset of breast carcinomas amplify the HER-2 gene without overexpression.

In the present study, a substantially higher percentage (55.5%) with IHC score 2+ cases was found to have gene amplification on FISH. Previous studies reported 6-25 per cent incidence of IHC 2+/FISH amplified cases. The ASCO/CAP guidelines³ reported an incidence of 23.9 per cent. Most of the studies including the present one showed an overall high concordance between IHC 3+/FISH amplified and IHC negative/FISH non-amplified groups.

In the present study 17 out of 18 IHC Positive (3+) cases showed gene amplification accounting to 94.4%, 5 out of 9 IHC equivocal (2+) cases showed gene amplification accounting to 55.5%, 5 out of 33 IHC negative (1+/0) cases showed gene amplification accounting to 15.2%. Panjawani et al³ reported an incidence of 12.2 per cent of gene amplification in IHC negative cases. Press *et al*⁶ have reported an incidence of 7.4 per cent of gene amplification in IHC negative cases. Present Study showed an incidence of 15.2% of gene amplification in IHC negative cases similar to other studies.

CONCLUSION

Breast cancer ranks as the second most common cancer for women in India and its incidence peaks between the ages of 40 and 50 yr, with a mean age of occurrence at 47 yr. Among the various prognostic and predictive factors of breast cancer, the most widely studied biomarker is the human epidermal growth factor receptor 2 (HER-2) gene, also referred to as ERBB2 or HER-2/neu, which is amplified in approximately 18-20 per cent of all breast cancers. Amplification of this gene is associated with the rapid progression of the disease, increased metastatic potential, increased resistance to tamoxifen and better response to anthracycline-based chemotherapy. The discovery of targeted therapy against the HER-2 gene in the form of the humanized anti-HER2 monoclonal antibody trastuzumab and HER-1/HER-2 dual receptor inhibitor, lapatinib, has brought forward an effective treatment modality for patients having the gene amplification.

In conclusion, in the present study both IHC and FISH were employed for determination of HER-2/neu status in invasive breast carcinoma. With most authorities agreeing upon FISH to be more reliable than IHC for determining the HER-2 status; FISH is an expensive, time consuming and labour intensive procedure, which requires training for interpretation. These constraints make IHC the most common method used for testing HER-2/neu.

References:

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: Estimates of world wide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010, in press.
2. Salmon DJ, Leyland-Jones B, Shak S et al. Use of chemotherapy plus a monoclonal antibody against C-erb-B2 for metastatic breast cancer that overexpresses C-erb-B2. *N. Engl. J. Med.* 2001; 344:783–792.
3. Tandon AK, Clark GM, Chamness GC, et al. C-erb-B2/neu oncogene protein and prognosis in breast cancer. *J Clin Oncol.* 1989;7:1120-1128.
4. Stanley P. L. Leong, Zhen-Zhou Shen, Tse-Jia Liu, Gaurav Agarwal, Tomoo Tajima, Nam-Sun Paik, Kerstin Sandelin, Anna Derossis, Hiram Cody, and William D. Foulkes. Is Breast Cancer the Same Disease in Asian and Western Countries?. *World J Surg.* 2010 October; 34(10): 2308–2324.
5. Panjwani P, Epari S, Karpate A, Shirsat H, Rajshekharan P, Basak R, et al. Assessment of HER2 status in breast cancer using Fluorescence in situ hybridization and immunohistochemistry: Experience of a tertiary cancer referral centre in India. *Indian J Med Res* 2010;132:287-94.
6. Press MF, Sauter G, Bernstein L, Villalobos IE, Mirlacher M, Zhou JY, et al. Diagnostic evaluation of HER-2 as a molecular target: an assessment of accuracy and reproducibility of laboratory testing in large, prospective, randomized clinical trials. *Clin Cancer Res* 2005; 11 : 6598-607.