Assessment of HER2NEU Gene Amplication by Flourescence in Situ Hybridization in Breast Carcinoma with Equivocal Findings on Immunostaining

Asra Taj, Ghulam Haider, Maliha Ashfaq, Mahnoor Ahsan, Khalil Ahmed, Wish Hal Sundar*

Department of Oncology, Jinnah Postgraduate Medical Center, Karachi Pakistan, *Department of Medicine, Civil Hospital, Karachi Pakistan

ABSTRACT

Objective: To determine the frequency of HER2/neu gene amplification by fluorescence in situ hybridization in Breast carcinoma patients with equivocal findings and to see its association with different clinicopathological features.

Study Design: Cross-sectional study.

Place and Duration of study: Medical Oncology Department, Jinnah Postgraduate Medical Center, Karachi, from Jan 2019 to Jan 2020.

Methodology: Total 166 females of age 22-72 years with diagnosis of invasive breast carcinoma which scored 2+ for HER2/neu by immunostaining were included in the study. Data regarding socio-demographics and clinicopathological features were noted for all the cases. Each patient was evaluated for estrogen and progesterone receptor status by immunostaining and score of 0 to 2 was deemed as negative while score 3 to 8 was deemed as positive. Patients having equivocal HER2/neu on immunostaining was further tested for gene amplification by fluorescence in situ hybridization.

Results: The mean age of study participants was 46.98±11.22 years (Range: 22-72 years). Out of 166(56.5%) showed gene amplification by fluorescence in situ hybridization. Statistically significant association was found between stage of tumor and HER2/neu (*p*=0.036).

Conclusion: HER2/neu gene amplification by fluorescence in situ hybridization is detected in more than half of patients with equivocal findings on IHC and HER2/neu is strongly correlated with stage of tumor.

Keywords: Human epidermal growth factor receptor 2, Gene amplification, Fluorescence in situ hybridization, Immunostaining.

INTRODUCTION

Worldwide approximately 2.1 million newly diagnosed cases of breast cancer (BC) have been estimated in 2018, contributing about 11.6% of the total cancer cases. In Asia the incidence of BC is less than Western countries.[1] The odds of BC have also increased in Pakistan, accounting for nearly one in nine females at risk of being diagnosed with BC and incidence rate is higher as compared to other Asian countries. Unfortunately, higher BC mortality in the country is, due to lack of awareness, late detection and poor access to adequate services.[2]

The human epidermal growth factor receptor 2 (HER2neu), a tyrosine receptor kinase protein is encoded by proto-oncogene located on long arm of chromosome 17.[3] Overexpression of HER2neu protein is usually a direct consequence of gene amplification except in 3-5% cases.[4] HER2neu overexpression or gene amplification occurs in 25-30% of breast carcinoma and is associated with poor prognosis due to rapid progression, high metastatic and recurrence rates.[5, 6] HER2 is the member of human epidermal growth factor receptor (HER/ERbB) family. Ligands for HER2 have not yet been identified, it undergoes dimerization that results in the auto phosphorylation of tyrosine residues of intracellular domain and activates a variety of signaling pathways.[3,5] The development of targeted HER2 gene therapy in the form of HER2 monoclonal antibody (trastuzumab, pertuzumab), antibody-drug conjugate (ado-trastuzumab emtansine, Famtrastuzumab deruxtecan) and tyrosine kinase inhibitors (lapatinib, neratinib) has culminated in a promising therapeutic modality for HER2neu positive BC patients.[5,19] HER2 targeted therapy have been shown to be active only in tumors that display real gene amplification. This is therefore of the utmost importance to identify the category of patients that would benefit from this modern type of therapy.[3, 6]

Correspondence: **Dr Asra Taj,** Department of Oncology, Jinnah Postgraduate Medical Center, Karachi Pakistan
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Although there are many techniques such as southern, northern and western blot and polymerase chain reaction-based analyses, mostly detection of the HER-2/neu status in breast carcinomas on tissue sample is done by fluorescence in situ hybridization (FISH) and/or immunohistochemistry (IHC).[7,8] IHC for detecting Her2neu is a simple, fast and cost-effective test compared to FISH but have a disparity in results due to variation in laboratory parameters. FISH is expensive and takes more time however; it is highly sensitive and specific for detecting HER2neu gene amplification and is more accurate.[9]

The strong agreement between IHC and FISH was observed for IHC scores of 1+ and 3+. IHC 2+ is equivocal score with variable agreement with FISH results.[3] Equivocal HER2neu on immunohistochemistry should be further tested for gene amplification by FISH so that we can make proper decision for patients who will need HER2 targeted therapy.[8] The purpose of the present research is therefore to evaluate the frequency of HER2 gene amplification by FISH in breast carcinoma patients with equivocal findings on IHC and to see its association with different clinico-pathological features.

METHODOLOGY

The cross-sectional study conducted at the Department of Medical Oncology, Jinnah Post-graduate Medical Center (JPMC), Karachi, Pakistan from Jan 2019 to Jan 2020. Sample size of 166 was estimated on Open epi sample size calculator by using statistics of concordance between immunochemistry and FISH as 76%, absolute precision as 6.5% and 95% confidence level.[5]

Inclusion Criteria: All females of age 22-72 years with confirmed diagnosis of invasive breast carcinoma which scored 2+ by IHC were included in the study.

Exclusion Criteria: Females with recurrent BC or who did not give consent were excluded from the study.

The study approval was obtained from ethical review committee of JPMC (F.2-81/2019-GENL/76806/JPMC) before initiation of study. Written informed consent was obtained from all the eligible patients. Data regarding socio-demographics and clinicopathological features were noted for all the included cases on pre-designed proforma. Each patient was evaluated for estrogen receptor (ER) and progesterone receptor (PR) by immunostaining and score of 0 to 2 was deemed as negative while score of 3 to 8 was deemed as positive. IHC was performed first on

all specimens and only equivocal cases were reflexed to FISH in accordance with the standard practice at the study institution. All HER2 IHC slides were re-evaluated by a breast pathologist who was blinded to the prior reported results and were classified according to the 2018 ASCO/CAP guidelines.[10] HER2/CEP17 ratio more than or equal to 2.0 by FISH was considered as positive and no equivocal results were included.

All data was entered and analyzed using SPSS version 23. The Mean±SD was reported for quantitative variables while frequency and percentage was reported for categorical or nominal variables. Association between Her-2/neu by FISH and clinicopathological features was assessed by using chi-square test. The p-value≤0.05 was taken as statistically significant.

RESULTS

Of 166 patients, the mean age of study participants was 46.98±11.22 years (Range: 22-72 years). Most of the females were residing in urban area (n=138, 83.1%) and were housewives (n=159, 95.8%). Majority of the females were Urdu speaking (n=84, 50.6%) followed by Sindhi (n=27, 16.3%). One sixty three (98.2%) females were married. Only 12 (7.2%) of the females had positive family history of BC (Table-I).

Table-I: Socio-demographic details of Study Sample (n=166)

Age (years)	Mean±SD
	46.98±11.22
	n(%)
Residence	
Urban	138(83.1)
Rural	28(16.9)
Occupation	
Housewife	159(95.8)
Student	2(1.2)
Working woman	5(3)
Ethnicity	
Sindhi	27(16.3)
Urdu	84(50.6)
Punjabi	18(10.8)
Pashto	14(8.4)
Balochi	13(7.8)
Others	10(6)
Marital Status	
Married	163(98.2)
Unmarried	3(1.8)
Family history of Breast Cancer	
No	154(92.8)
Yes	12(7.2)

About 70 (42.2%) females had stage III, 117 females (70.5%) had grade II of tumor and 160 (96.4%) females had invasive ductal carcinoma (IDC). Half of

the females had right side carcinoma (n=83, 50%) followed by left (n=79, 47.6%) and both sides (n=4, 2.4%). About 126 (75.9%) females had lymph nodes involved and among them 84 (66.7%) had more than and equal to 3 lymph nodes involved. Out of 166 females, 111 (66.9%) had positive ER status and 94 (56.6%) had positive PR status (Table-II).

Table-II: Clinicopathological findings of Study Sample (n=166)

	n(%)
Stage	
I	6(3.6)
II	50(30.1)
III	70(42.2)
IV	40(24.1)
Grade	
I	10(96)
II	117(70.5)
III	39(23.5)
Histology	
Invasive ductal carcinoma	160(96.4)
Invasive lobular carcinoma	2(1.2)
Mucinous	2(1.2)
Others	2(1.2)
Laterality	
Right	83(50)
Left	79(47.6)
Both	4(2.4)
Lymph node	
Positive	126(75.9)
Negative	40(24.1)
Number of lymph nodes	
<3	42(33.3)
=>3	84(66.7)
Progesterone receptor	
Positive	94(56.6)
Negative	72(43.4)
Estrogen Receptor	
Positive	111(66.9)
Negative	55(33.1)

Out of 166(56.5%) showed HER2neu gene amplification by FISH. The HER2/neu was compared with clinicopatho-logical features. The significant association was found between stage of tumor and HER2/neu (*p*=0.036). No significant association was found between HER2/neu and grade (*p*=0.658), lymph nodes (*p*=0.465), number of lymph nodes (*p*=0.85), ER (*p*=0.342) and PR status (*p*=0.807) Significant finding (*p*≤0.05) (Table-III).

DISCUSSION

Evaluation of the Her-2/neu gene status has been part of the main data collection for pathological reporting of invasive breast cancer.[11] Despite some evidence that FISH experiments predict therapeutically relevant Her-2/neu status more reliably, the method of primary immunohistochemistry screening with additional FISH molecular validation is commonly used. However several centers prescribe an initial review in cases of invasive breast cancer with IHC complemented by FISH where the findings of IHC are inaccurate. Several studies have shown that IHC score 2+ Her-2/neu is always followed by true gene amplification, whereas others have recorded considerably higher frequencies.[6, 11]

Table-III: Association of HER-2 Status with Clinicopathalogocal Features (n=166)

	HER2neu gene Amplification by FISH		*p*-value
	Detected	Not detected	
Grade of tumor			
I	7 (70%)	3(30%)	
II	64 (54.7%)	53(45.3%)	0.658
III	23 (59%)	16 (41%)	
Stage of tumor			
I	4(66.7%)	2(33.3%)	
II	35(70%)	15(30%)	0.036
III	31(44.3%)	39(55.7%)	
IV	24(60%)	16(40%)	
Lymph nodes			
Positive	69(54.5%)	57(45.2%)	0.465
Negative	25(62.5%)	15(37.5%)	
Number of lymph nodes			
<3	24(57.1%)	18(42.9%)	0.85
≥3	45(53.6%)	39(46.4%)	
Estrogen Receptor			
Positive	60(54.1%)	51(45.9%)	0.342
Negative	34(61.8%)	21(38.2%)	
Progesterone receptor			
Positive	54(57.4%)	40(42.6%)	0.807
Negative	40(55.6%)	32(44.4%)	

Our study shows that on histological basis, invasive ductal carcinoma of breast is the most common type whereas invasive lobular carcinoma, mucinous and other types of breast cancers are less common. Recently, few studies conducted in Pakistan have also validated similar findings,[12-14] 66.9% had ER positive and 56.6% had PR positive. The findings are slightly less than a study which showed 71% cases had ER and PR positive tumors.[15] The baseline characteristics of present study sample also ascertain that grade 2 and stage 3 breast cancer are found commonly. The findings are also in concurrence with other studies.[13-15] In the current study, majority of the patients presented with lymph node involvement. Saleh *et al*. reported that in 774 breast cancer patients, 35.5% had axillary lymph node involvement at the time of diagnosis.[16]

The present study results elucidate that more than half of the patients of breast cancer presented with equivocal HER2neu on immunostaining had gene amplification detected by FISH which is greater than that detected by Sudha S murthy.[17,18] As the assessment of HER2neu status is integral part in management of breast carcinoma, so it should be properly evaluated.[19] The testing of HER2neu gene has become quit complex since the development of different techniques. The majority of HER2 testing is performed using a combination of IHC screening followed by FISH for IHC equivocal cases.[20] Fan *et al*. reported that equivocal Her-2/neu on IHC showed variable agreement with FISH results. Even though FISH is a more complex and expensive procedure, it should be considered the method of choice for equivocal cases by IHC that are not always accompanied by true gene amplifications.[21] With development of new HER2 targeted agents that are expensive and have side effects, so it is very important to correctly diagnose patient who have HER2neu gene amplification.

With regards to association among baseline characteristics of breast cancer patients with Her-2/neu gene amplification, there was no statistically significant relation existed with age, grade, lymph nodes. The similar results were found in other studies which shown no association of Her-2/neu with age, grade and lymph nodes.[4,11,22] However, many studies reported statistically significant association between HER-2/NEU and ER, PR status whereas the present study results are in disagreement with previous study findings by Panjwani *et al*. and Mostafa *et al*.[6,11] Only statistical significance was found between stage of the tumor and Her-2/neu status which is in disagreement with previous study conducted by Raquel *et al*.[23] In another study by Siadati *et al*. observed that HER-2/neu was inversely related to ER and PR expression (p=0.0001) and statistically significant correlation was found between HER-2/neu expression and lymph node metastasis (p=0.03).[24]

It was a cross sectional study so we could not measure the cause-effect relationship. Further sample size was also small therefore results could not be generalized for whole population. Non-probability sampling technique was applied in which equal chance was not given all eligible participants. However, studies with larger sample size are needed to be done to further validate the results of the current study.

CONCLUSION

Her-2/neu gene amplification by FISH is detected in more than half of patients with equivocal results on IHC and HER2neu is strongly correlated with stage of tumor.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AT & GH: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

MA & MA: Conception, study design, drafting the manuscript, approval of the final version to be published.

KA: & WHS: Critical review, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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