RESEARCH COMMUNICATION

Status of HER2 Amplification, Polysomy17 and Histopathological Features of 425 Pakistani Breast Cancer Patients

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Abstract

HER2 gene amplification in invasive breast cancer is a robust predictive marker for response to transtuzumab therapy. This study was undertaken to measure concordance between immunohistochemistry (IHC) and FISH for HER2 gene amplification in invasive breast tumors, as well as the presence of polysomy 17 and possible correlation with demographics and histopathological variables, including ER and PR positivity. A total of 425 cases of infiltrating carcinoma of breast (99% IDC-NOS) were studied. HER2 over expression was tested by IHC and FISH methods. Association between IHC and FISH in both subsets was calculated by amplification ratio including polysomy 17. Out of 425 specimens, 128 (30%) were positive for HER2 amplification by FISH test, whereas only 78 (24%) tumors with 2+ expression showed amplification. In contrast, 39 (74%) demonstrated 3+ IHC score and HER2 gene amplification. The histological variables including tumor size, tumor type, and lymph node involvement did not influence the outcome of FISH analysis. The ER and PR status showed significantly greater positivity in patients negative for HER2 amplification. Polysomy 17 was detected in 23.7% patients and was positively associated with ER and PR expression (P= <0.05). Our study showed a concordance of 24% between 2+ IHC and FISH amplification, while in 3+ IHC cases the concordance was 74%. Significant links of HER2 amplification was seen with ER andPR negativity and higher tumor grade. In addition, non-significant correlations were noted with other variables like tumor type, size and lymph node status.

Keywords: Breast cancer - HER2 - gene amplification - polysomy 17 - FISH - immunohistochemistry - Pakistan

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Introduction

Breast cancer is one of the most common cancers occurring among women throughout the world, and is the leading cause of death from cancer. Globally, it averages more than 1.1 million new cases, where developed countries face 55% of the burden; the incidence is rapidly catching up in the developing world as well (Parkin et al., 2005). In Pakistan like other Asian countries, breast cancer among females is frequently reported at an advanced stage, which is most likely due to lack of awareness and early screening programs (Ahmad, 1991; Naeem et al., 2008).

Being a heterogeneous disease, breast cancer is characterized by multiple subtypes as revealed by differential gene expression profiles. Its diverse nature points towards the involvement of multiple prognostic and predictive tumor markers (Turaga et al., 2010). Epidermal growth receptor 2, commonly known as HER2 is a crucial breast cancer oncogene, which is located on the long arm of chromosome 17 and encodes a 185-KDa cell surface receptor with tyrosine kinase activity (Akiyama et al., 1986; Slamon et al., 1989). Although, early studies have claimed over-expression of HER2 in approximately 30% of breast cancer cases, but a revised estimate suggested lower frequency in the general population. For instance in 2004, Yaziji et al reported approximately 18% HER2 expression in a US study (Yaziji et al., 2004). Similarly, a study carried out by Owen et al also reported HER2 over expression in 20% biopsy samples tested by IHC and FISH methods (Owen et al., 2004). HER2 receptor is normally expressed at a low level in epithelial cells where it plays an important role in proliferation and differentiation through activation of subcellular signal transduction pathways. High level of HER2 expression is considered to be a strong predictive marker for selecting trastuzumab for therapy in patients with invasive type (Slamon et al., 2001; Ross et al., 2004). Clinical trials have proven that trastuzumab is effective as monotherapy and also in combination with chemotherapy for treating HER2 over expressing breast tumors (Baselga et al., 1996; Vogel et al., 2002; Garnock-Jones, 2010). In essence, evaluation of HER2 over expression has become an important tool for breast cancer management.

The aim of the present study was to retrospectively

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evaluate breast cancer biopsies for HER2 gene amplification, identify prevalence of polysomy 17 and associate with histopathological characteristics in a large cohort of Pakistani patients.

Methods

Specimens

Biopsy specimens from patients unequivocally diagnosed as invasive breast carcinoma between 2007 and 2010 at the Histopathology Laboratory of the Aga Khan University Hospital were included in this study. Overall 425 cases were studied; all were primary tumors collected subsequent to mastectomy, lumpectomy or as core biopsy and brought to the laboratory in 10% buffered formalin. For statistical analysis, clinicopathological features including histological type, tumor size, histological grade ascertained by modified Blooms and Richardson's grading system, lymphocytic infiltration, hormonal receptor status and patients' age were obtained from patients' files. All tissue specimens were first tested for HER2 status by IHC and those that were 2+ and a subset of 3+were screened by FISH technique.

Immunohistochemistry

HER2 protein expression in tumor specimens was analyzed by Hercept test according to the manufacturer's recommended protocol (DAKO, Carpentaria, CA). For antigen retrieval 3-4 μm thick sections were transferred in the retrieval solution (Tris-EDTA pH 9) and then placed in microwave oven at 450 W for 20 minutes followed by washes in tap water. Rabbit polyclonal antibody (clone: A0485, DAKO) was applied at a dilution of 1:100 for 30 minutes and subsequently detected by Envision System (DAKO). Both positive and negative controls provided in the kit were included with each batch of samples. The scoring criteria for HER2 staining were followed as suggested by 'College of American Pathologists' (CAP) guideline (Wolff et al., 2007). Results from IHC were reported in categories such as 0, 1+, 2+or 3+ scores.

Fluorescence In Situ Hybridization

For the analysis of HER2 gene status, FISH assay was performed using FDA approved PathVysion system (Abbott, Downer Grove, IL). Briefly, 4-5 μm thick sections were sliced from each tumor block and placed on charged glass slides. After paraffin removal, sections were dehydrated and incubated in 2mM NaCN for 20 minutes and later held in pepsin for an average 30 min followed by denaturation in formamide solution. To each section 10 μl of DNA probe cocktail containing CEP17 (green signal) and LSI HER2 (red signal) was applied, coversliped and sealed for overnight hybridization at 37 °C in a humidified chamber. Afterwards, slides were washed, counter stained with DAPI and observed for probe signals using a Nikon epi-fluorescent microscope (Nikon, Tokyo, Japan). For scoring, invasive component of the tumor was selected on H&E stained sections. Red and green signals emanating from the stained sections were counted and interpreted according to the ASCO guidelines (Wolff et al., 2007). A ratio of HER2 to CEP17 of ≥ 2.2 was considered as HER2 amplified whereas ≤ 1.8 was interpreted as nonamplified and between 1.8 and 2.2 as equivocal. According to Torrisi and colleagues, polysomy of chromosome 17 was characterized as ≥ 3 green signals per cell (Torrisi et al., 2007).

Statistical Analysis

Statistical analysis was carried out using SPSS version 19.0 for windows (SPSS Inc., Chicago, IL). Independent t-test was used to compare means. Relationship between HER-2 FISH status versus variables including, tumor grade, ER status, PR status, lymph node and IHC score was assessed using univariate logistic regression. Moreover, odd ratio(OR) along with 95% confidence intervals (CI) were computed for each variable. All statistical tests were two sided and an association was considered significant when P value was less than 0.05.

Results

In the present study, 425 female invasive breast cancer tissue samples were initially examined for HER2 by IHC and specimens demonstrating 2+ and some 3+ scores were evaluated by FISH analysis. Figure 1 shows representative images of HER2 amplified, non amplified and polysomy 17 positive specimens. Out of 425 specimens 128 (30%) were positive for HER2 amplification by FISH test, whereas 78/324 (24%) tumors with 2+ expression showed amplification. In contrast, 39/53 (74%) demonstrated 3+ IHC score and HER2 gene amplification.

The average HER2 amplification ratio was 2.9. Overall, mean age of women was 55 years (range 26-88 years). There was no difference in the average age of patients between carriers of HER2 positive or negative tumors. Table 1 compares histological characteristics among HER2 amplified and non-amplified groups. The histological variables including tumor size, tumor

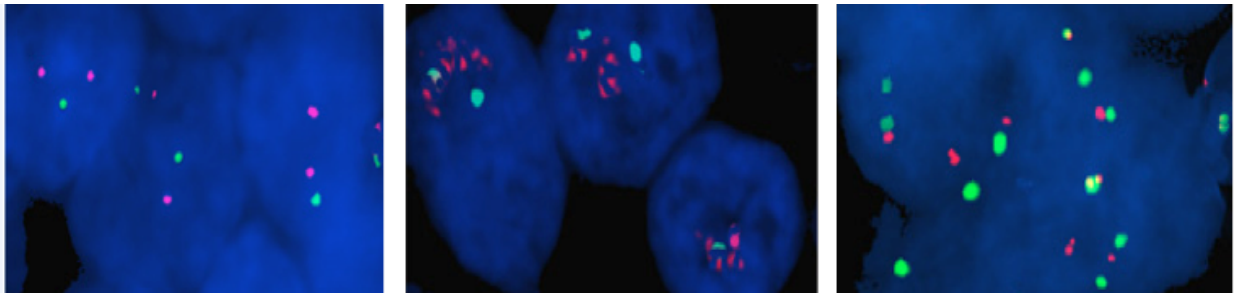

Figure 1. Representative Photographs of FISH Assay Results. (a) Normal (two red signals and two green signals); (b) Strong Amplification (multiple red signals and two green signals) (c) Polysomy 17 (multiple red and green signals)

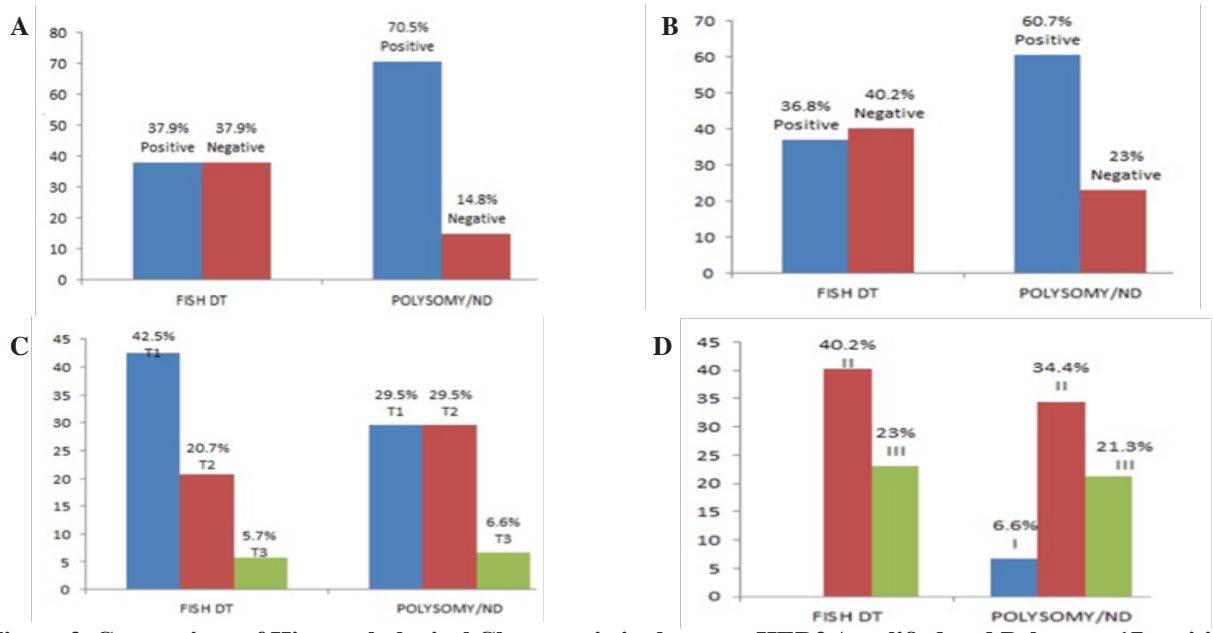

Figure 2. Comparison of Histopathological Characteristics between HER2 Amplified and Polysomy 17 positive (Amplification Eegative) Patients. Subsets a-d, show associations between Polysomy 17 and ER, PR status, tumor size and tumor grade (DT, detected, ND, not detected)

Table 1. HER Gene Amplification Was Compared Against a Variety of Histopathological Variables

Features	HER2 Amplified	HER2 non-Amplified	P value	Odds Ratio (95% CI)
Mean age+SD	54+11.55	55.7+12.22	0.408	0.992(0.97,1.01)
Tumor Type	N(%)	N (%)	0.190	1.973 (0.72,5.40)
IDC-NOS	95(74.2)	187(61.3)		
Lobular	1 (0.8)	8 (2.6)		
Papillary	3 (1.0)			
Mix Ductal and lobular	1 (0.3)			
Mucinious	5(1.7)			
Poor differ Ca	1(0.8)			
Invasive cribriform		1(0.3)		
Unknown	31(24.2)	93(31.3)		
Grade			0.006	
I	1(0.8)	12(4)		1(reference)
II	53(41.4)	128(43.1)		4.97(0.63,39.1)
III	32(24.4)	39(13)		9.85(1.21,79.8)
Unknown	42(32.8)	118(39.7)		
ER Status				
Positive	50(39.1)	173(58.2)	<0.001	0.35(0.213,566)
Negative	50(39.1)	60(20.2)		1(reference)
Unknown	28(21.4)	64(21.5)		
PR Status		<0.001		
Positive	51(39.8)	182(61.3)		0.31 (0.18,512)
Negative	47(37.4)	52(17.5)		1(reference)
Unknown	30(23.4)	63(21.2)		
LN Involvement			0.098	
No(ve+)	13(10.2)	50(16.8)		
N1(1-3)	17(13.3)	29(9.8)		2.26(0.96,5.30)
N2(4-9)	8(6.3)	17(5.7)		1.81(0.64,5.11)
N3(>10)	12(9.4)	15(5.1)		3.08(1.16,8.15)
Unknown	78(60.9)	186(62.6)		
IHC Score				
2+	78(60.9)	246(82.8)	>0.001	0.104(0.53,205)
3+	39(30.5)	13(4.4)		1(reference)
Unknown	11(8.6)	38(12.8)		
Total	128	297		

Table 2. Correlation between IHC and FISH for the Assessment of HER2 Status

	IHC Score			
FISH Status	Unknown N(%)	2+ N(%)	3+ N(%)	Total
FISH Positive	11 (22)	78 (24)	39 (74)	128
FISH Negative	38 (77.5)	247 (76.2)	13 (24.5)	297
Total	49	324	53	425

type, and lymph node involvement did not influence the outcome of FISH test. Major tumor types identified in patients included 'infiltrating ductal carcinoma, not otherwise specified (IDC-NOS)', which was equally distributed between the two groups, whereas papillary, mixed ductal and lobular and mucinious were found only in the non-amplified group. On the contrary, poorly differentiated and invasive cribriform were observed in the HER2 amplified patients. Only 1% cases classified in the low grade category were FISH positive, conversely, twice as many high grades tumors were FISH positive. Patients with tumor grade III were clustered more in the HER2 amplified group, which was statistically significant ($p < 0.007$).

Compared to HER2 amplified tumors, immunohistochemically determined ER and PR positivity was concentrated more in the HER2 non-amplified group and it was statistically significant ($p < 0.001$). A significant number of patients showed discrepancy between FISH and IHC results, which at times were due to false positivity in IHC results either for reasons of increased background level or over interpretation of staining pattern (Table 2). Another major reason for IHC and FISH discrepancy was polysomy 17 (>3 CEP17 signals/cell), which was detected in 23.7% of patients, conversely, monosomy 17 was found in 20% of patients. Mean CEP 17 signal/nuclei in polysomy 17 patients was four and average ratio 2.85. Figure 2 illustrates that there was no statistical difference

in tumor grade, size and ER status between the HER2 amplified and polysomy 17 positive groups. However, ER and PR expression was significantly higher in the polysomy 17 positive patients ($p < 0.05$).

Discussion

As a prognostic and predictive marker, assessment of HER2 gene amplification status using FISH in IHC 2+ breast tumors has become a standard practice (Slamon et al., 1989; Offersen et al., 2008). HER2 gene amplification and receptor over expression are known to be associated with poor prognosis. Immunohistochemistry is commonly used for the estimation of HER2 receptor expression in target tissues, whereas HER2 gene amplification is measured by FISH and is considered to be the gold standard. In the present study, 425 breast cancer cases from excised tumor were investigated for clinicopathological characteristics including hormonal receptor status and compared with HER2 amplified and non-amplified groups. Several published studies have shown strong correlation between assessment of HER2 receptor expression by IHC and gene amplification by FISH in cases with 0/1+ and 3+ IHC scores. In comparison, our study has also demonstrated similar correlation between IHC 3+ score and FISH positive cases, the concordance rate was 74%. On the contrary, 13 cases demonstrated 3+ positivity by IHC but remained FISH negative. In the published literature, the concordance rate of FISH and IHC ranges between 49% and 100%. For instance, Hammock and colleagues reported 49% concordance between IHC 3+ and FISH positivity; in their study only 22 out of 45 tumors with IHC 3+ exhibited HER2 gene amplification by FISH (Hammock et al., 2003). On the contrary, high concordance (81%) between IHC 3+ and FISH was shown by Prati et al in 199 invasive breast cancer cases (Prati et al., 2005). As illustrated in this study, IHC 2+ cases categorized as equivocal have shown only 24% concordance with FISH assay. These results are comparable to 25.7% association between IHC 2+ and FISH positivity reported by Prati and colleagues. Some of the reasons for disparity between IHC2+ and FISH results included presence of polysomy 17, which was observed in approximately 23.7% of cases and was noted in higher proportion in HER2 amplified tumors. Other plausible explanations are intramural heterogeneity, variation in fixation, tissue processing and tumor selection. As depicted in this study, HER2 amplification was observed in 128 (30%) cases, which is at the higher end of HER2 over expression range 18-30% reported in the published studies (Slamon et al., 1987; Al-Ahwal, 2006; Offersen et al., 2008) . Similar to worldwide pattern, IDC–NOS was overwhelmingly the most common histological subtype in our patients' cohort. It is an established observation that HER2 positive status is associated with increasing tumor grade (Tsuda et al., 1990; Vijver, 2002; Taucher et al., 2003). Similarly in our study only one case (0.8%) of low grade tumor was FISH positive, whereas grade III tumors were clustered more in HER2 amplified

compared to non-amplified group (24.4% versus 13%). In addition, other histopathological variables like tumor size, lymph node involvement did not show any correlation.

According to published observations HER2 amplification is inversely correlated with ER status (Konecny et al, 2003). Contrary to published reports, our data demonstrated converse relationship, as ER positive and negative expression were equally observed in our HER2 positive patients. The reason of this distribution could be age of patients reported in our study. The mean age of breast cancer patients was 55 years and majority of the patients were above 50 year of age (58%), which directly correlate with ER over expression (Eppenberger et al., 2002). Furthermore, level of HER2 gene amplification is inversely associated with ER and PR expression (Konecny et al., 2003). Similarly, in our study level of HER2 amplification was low (mean ratio 2.85). On the other hand, non amplified group showed higher number of ER and PR positives and the difference was statistically significant ($P<0.001$). In the present cohort, polysomy 17 was detected in 32.0% of HER2 amplified tumors averaging four CEP signals/nuclei and the average ratio was 2.95. These findings are lower compared to Wang and colleagues who reported greater than 50% breast tumors positive for polysomy 17 (Wang et al., 2002). The authors had suggested that the correlation between HER2 protein expression and chromosome 17 copy number could be influenced by high polysomy 17. These tumors are also noted as a source of discrepancy between 3+ IHC and FISH results. Furthermore, we have documented 23% polysomy 17 in HER2 non-amplified and IHC 2+group. In a small subset of patients (3 cases) who were HER2-nonamplified and IHC3+, polysomy 17 positivity was a plausible explanation for strong over expression of HER2 receptors. Similarly other studies have also corroborated that polysomy 17 is a major factor in strong HER2 protein over expression in 3+ nonamplified cases (Varshney et al., 2004; Ma et al., 2005). In summary, higher concordance was observed between IHC 3+ and HER2 gene amplification. Significant correlation of HER2 amplification was seen with ER, PR negativity and higher tumor grade. In addition, polysomy 17 was reported (32.0%) in HER2 amplified and (20.0%) non amplified tumors respectively. Non-significant correlation was seen with other variables like tumor type, size and lymph node status.

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